FIG. 1A

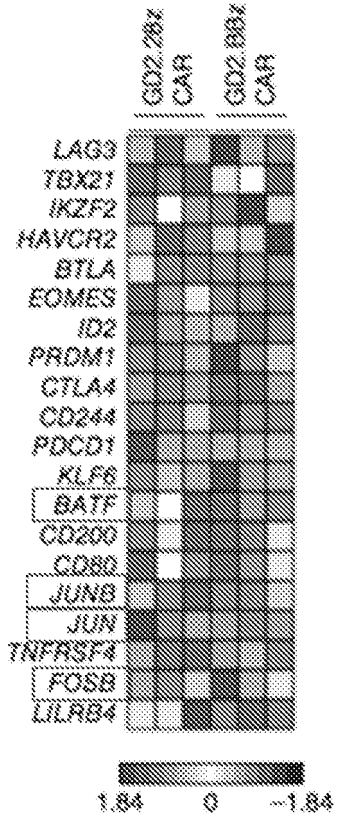

GD2.28z CAR
GD2.BBz CAR
LAG3
TBX21
IKZF2
HAVCR2
BTLA
EOMES
ID2
PRDM1
CTLA4
CD244
PDCD1
KLF6
BATF
CD200
CD80
JUNB
JUN
TNFRSF4
FOSB
LILRB4
1.84
0
−1.84

FIG. 1B

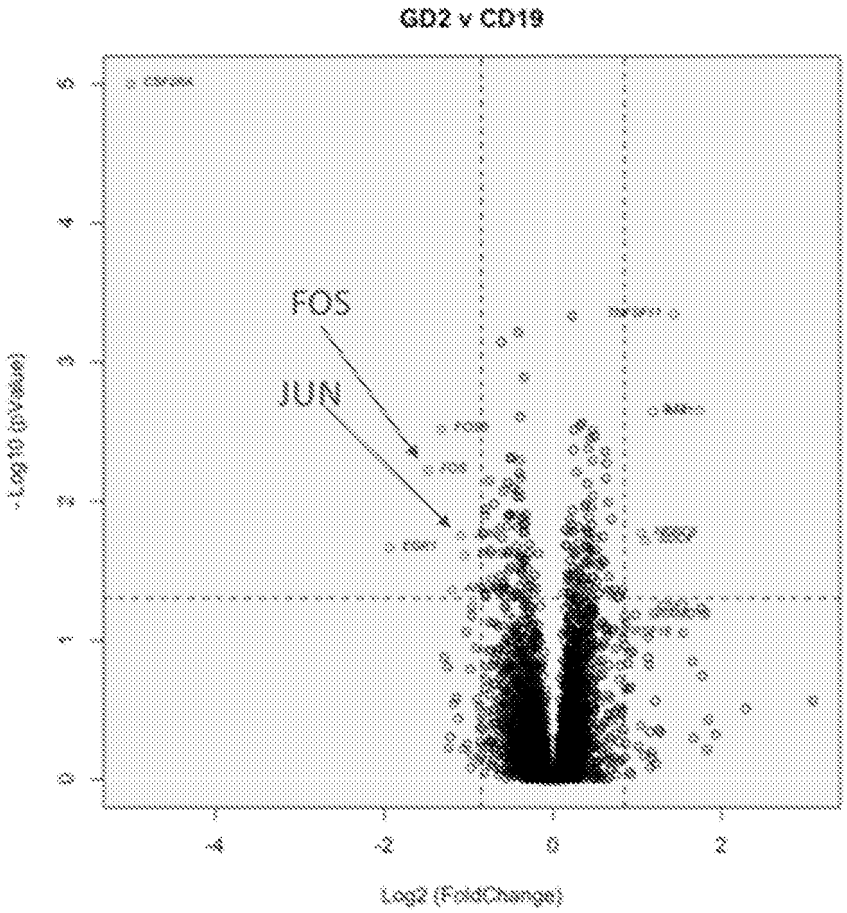

GD2 v CD19
FOS
JUN
-Log10 (pValue)
Log2 (FoldChange)

FIG. 2A



FIG. 2B

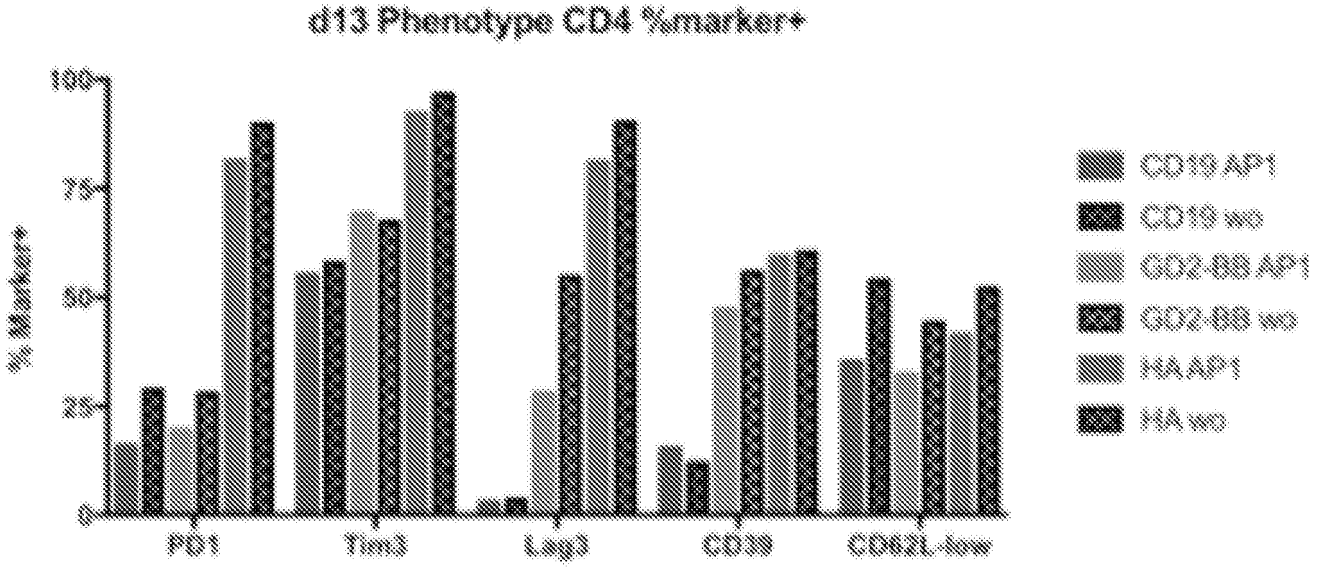


FIG. 2C

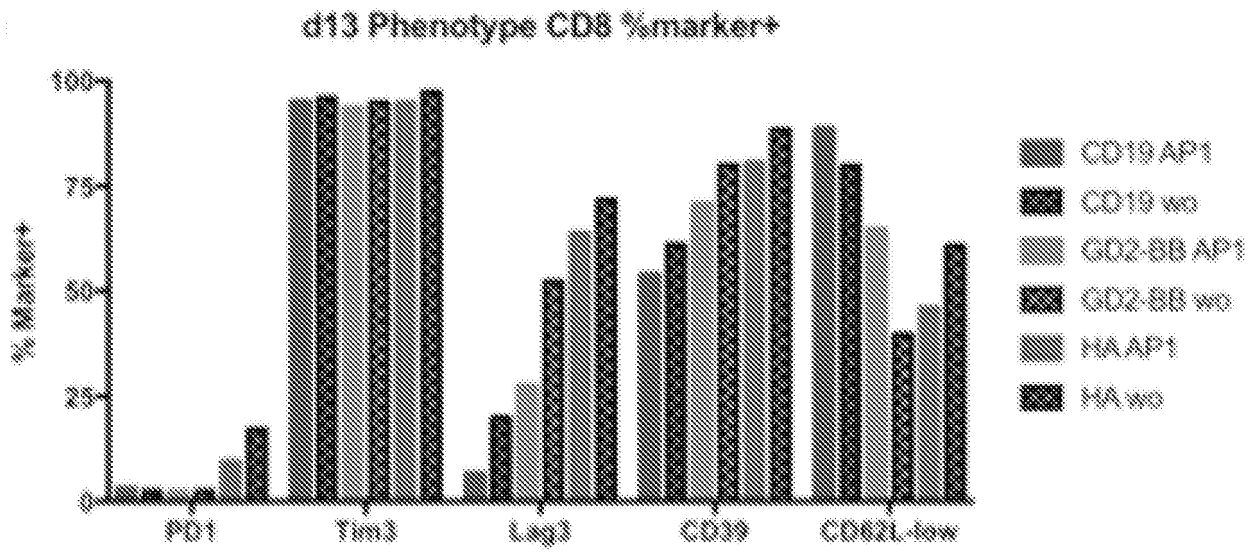


FIG. 2D

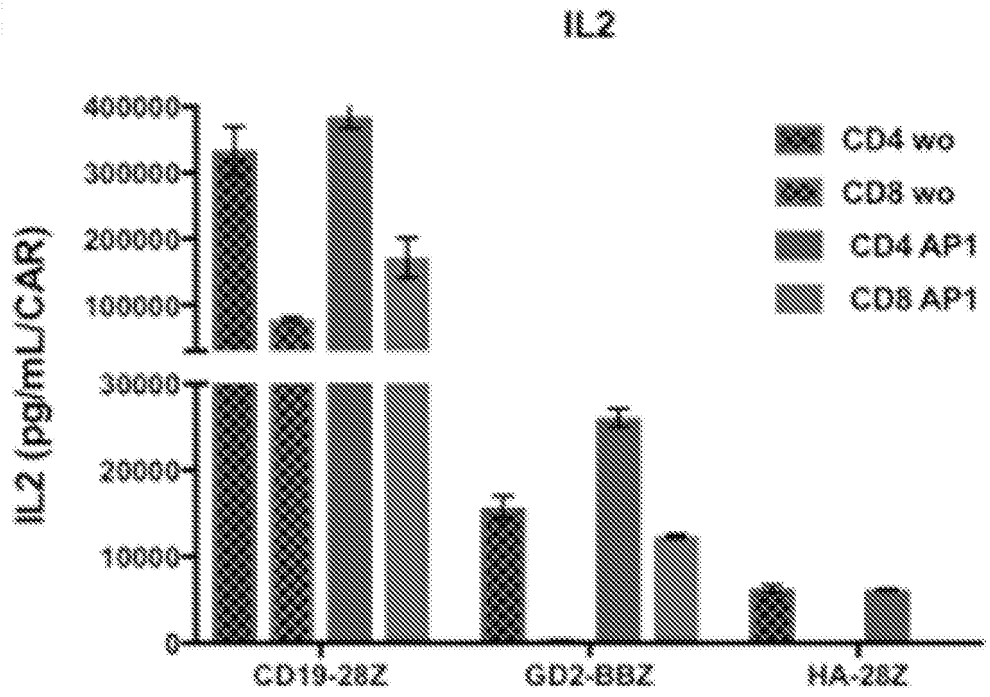

IL2
IL2 (pg/mL/CAR)
400000
300000
200000
100000
30000
20000
10000
0
CD4 wo
CD8 wo
CD4 AP1
CD8 AP1
CD19-28Z
GD2-BBZ
HA-28Z

FIG. 2E

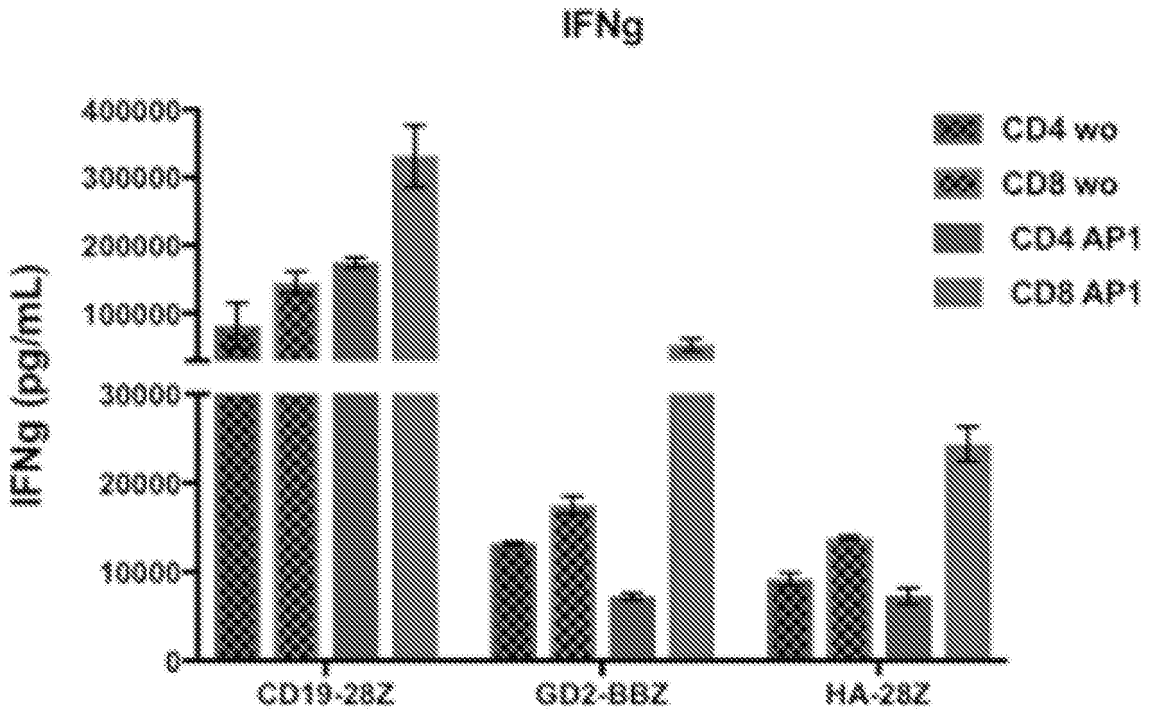

IFNg
IFNg (pg/mL)
400000
300000
200000
100000
30000
20000
10000
0
CD4 wo
CD8 wo
CD4 AP1
CD8 AP1
CD19-28Z
GD2-BBZ
HA-28Z

FIG. 3A



FIG. 3B

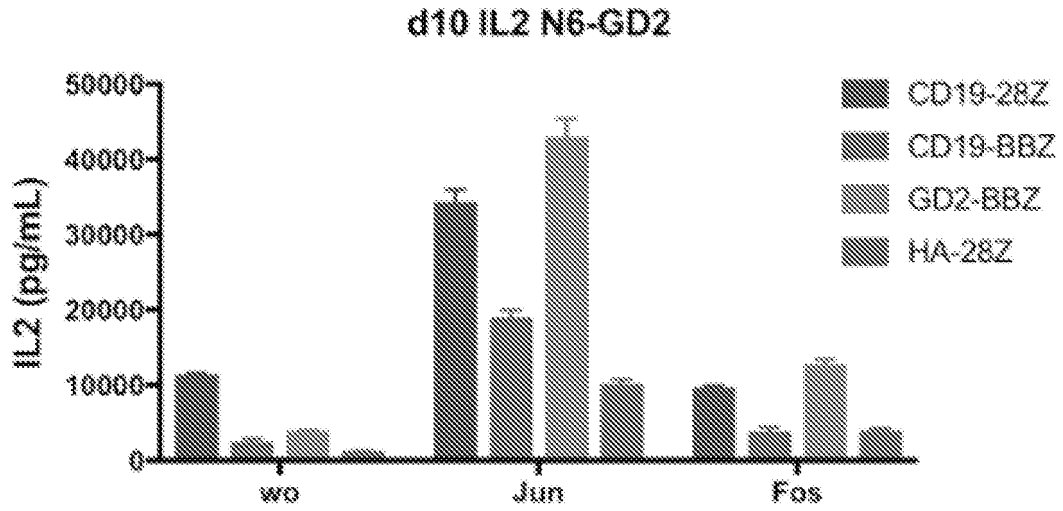


FIG. 3C

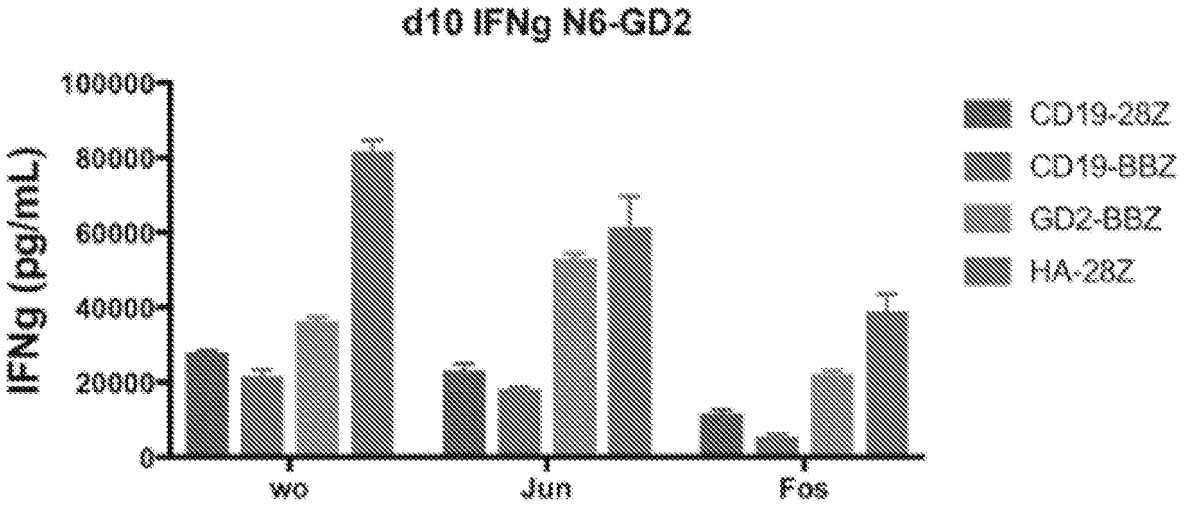


FIG. 3D

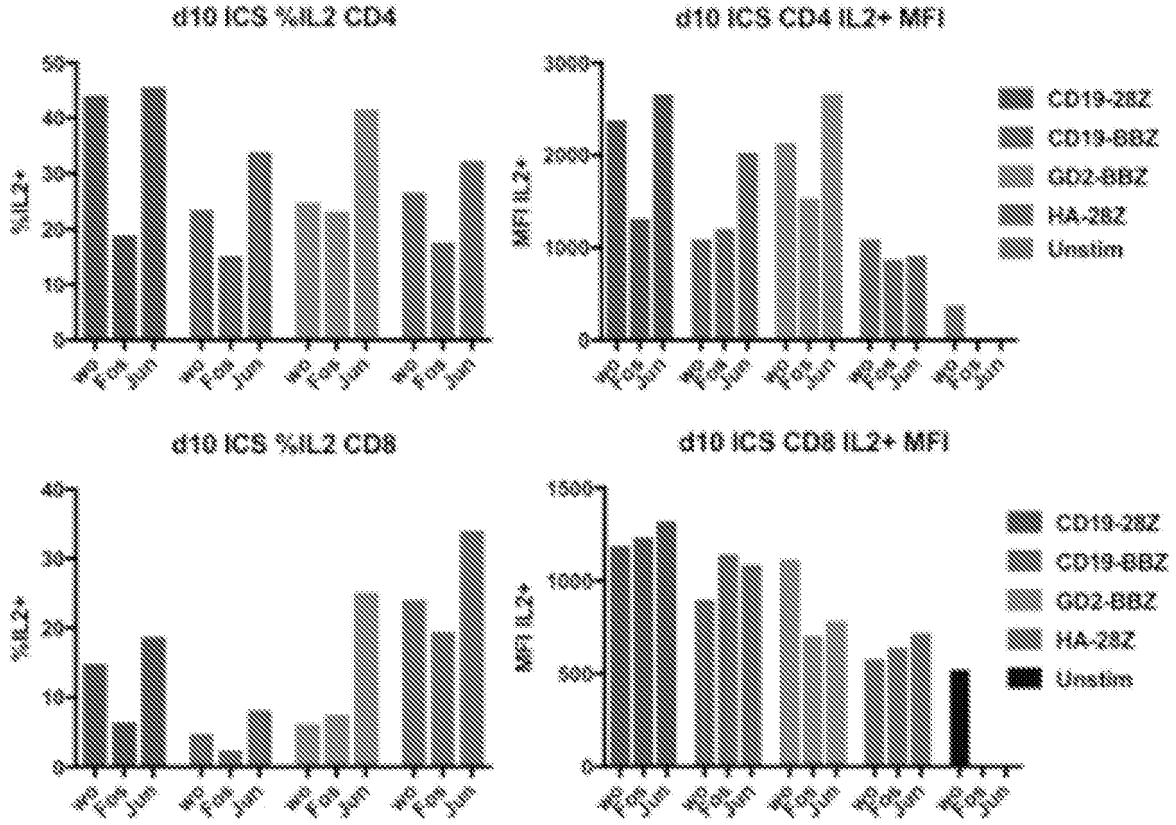

d10 ICS %IL2 CD4
%IL2+
d10 ICS CD4 IL2+ MFI
MFI IL2+
CD19-28Z
CD19-BBZ
GD2-BBZ
HA-28Z
Unstim
d10 ICS %IL2 CD8
%IL2+
d10 ICS CD8 IL2+ MFI
MFI IL2+
CD19-28Z
CD19-BBZ
GD2-BBZ
HA-28Z
Unstim

FIG. 3E

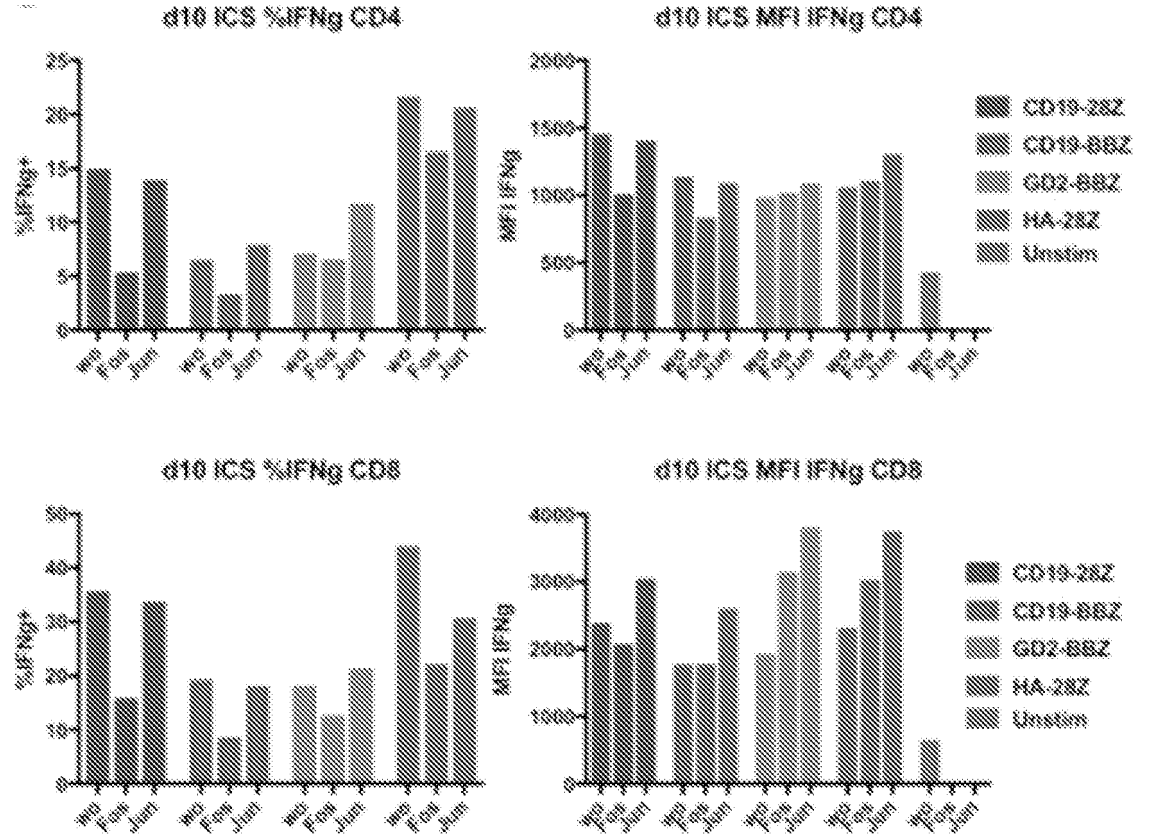
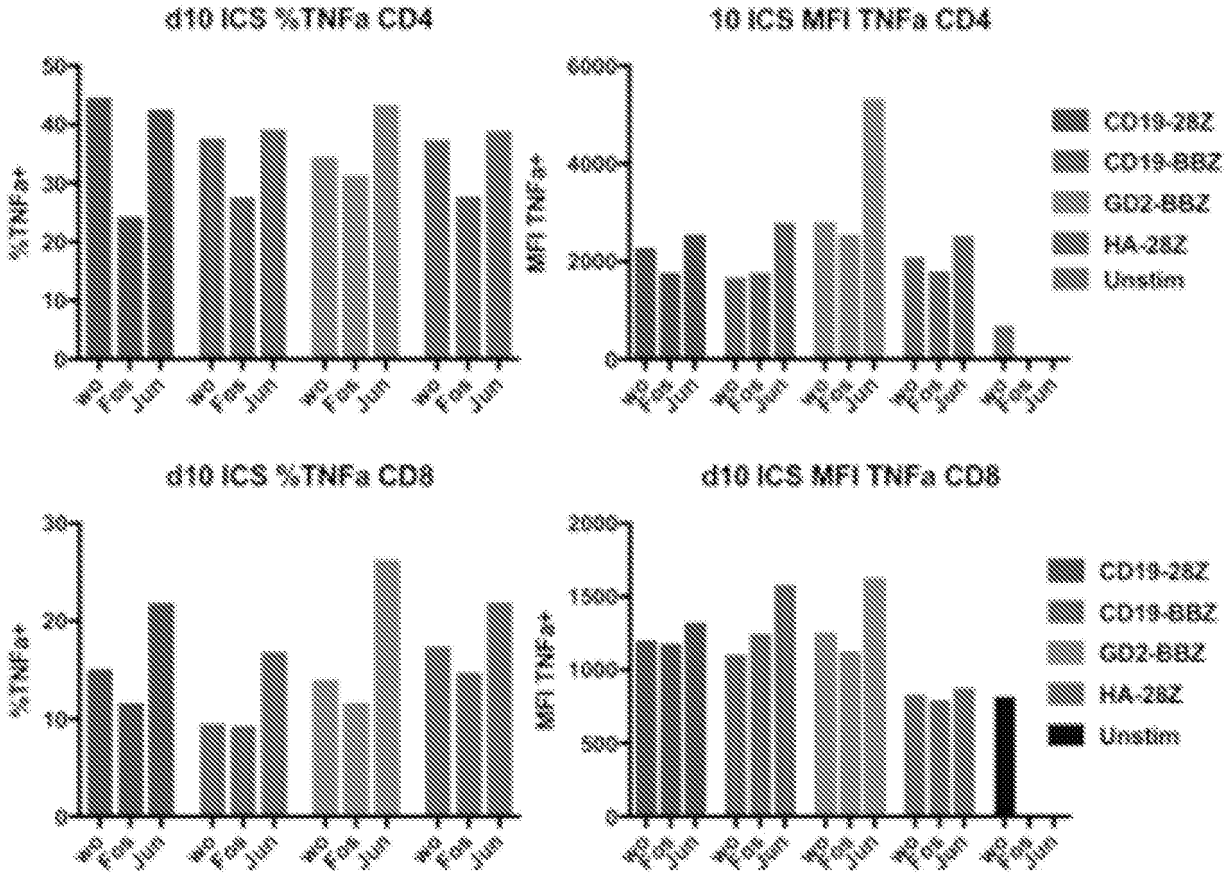

d10 ICS %IFNg CD4
%IFNg+
d10 ICS MFI IFNg CD4
MFI IFNg
CD19-28Z
CD19-BBZ
GD2-BBZ
HA-28Z
Unstim
d10 ICS %IFNg CD8
%IFNg+
d10 ICS MFI IFNg CD8
MFI IFNg
CD19-28Z
CD19-BBZ
GD2-BBZ
HA-28Z
Unstim

FIG. 3F


d10 ICS %TNFa CD4
10 ICS MFI TNFa CD4
d10 ICS %TNFa CD8
d10 ICS MFI TNFa CD8
%TNFa+
MFI TNFa+
CD19-28Z
CD19-BBZ
GD2-BBZ
HA-28Z
Unstim


MSGV
cJun-P2A-CAR

FIG. 4A

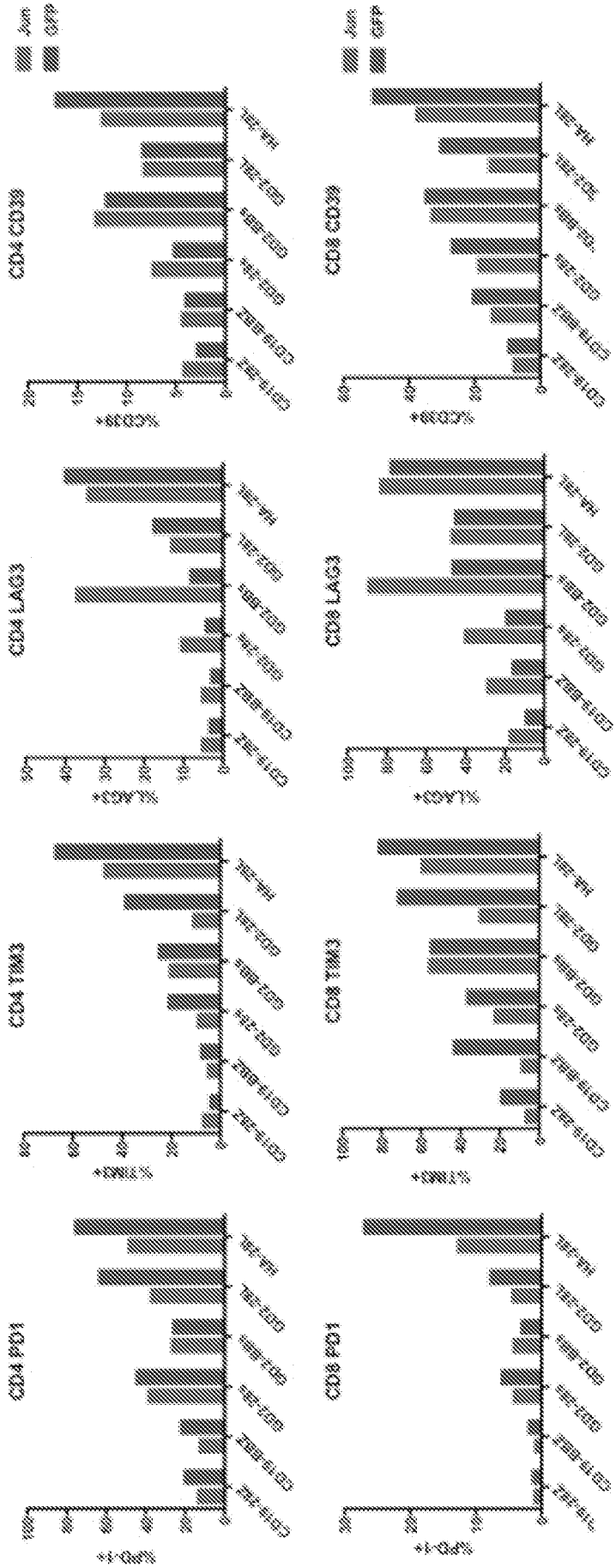

Δm
GFP
CD4 CD39
CD8 CD39
CD4 LAG3
CD8 LAG3
CD4 TIM3
CD8 TIM3
CD4 PD1
CD8 PD1

FIG. 4B

FIG. 4C

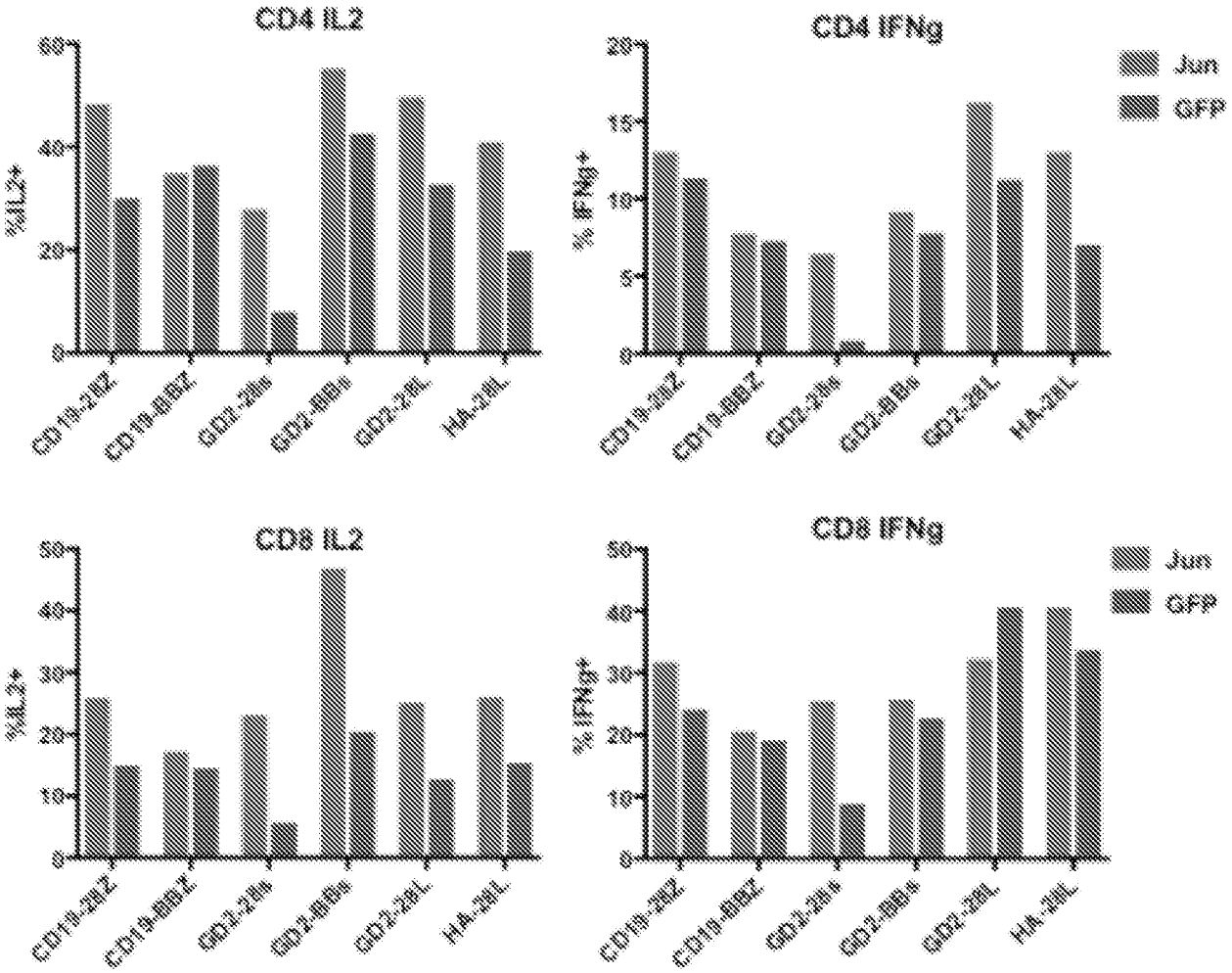
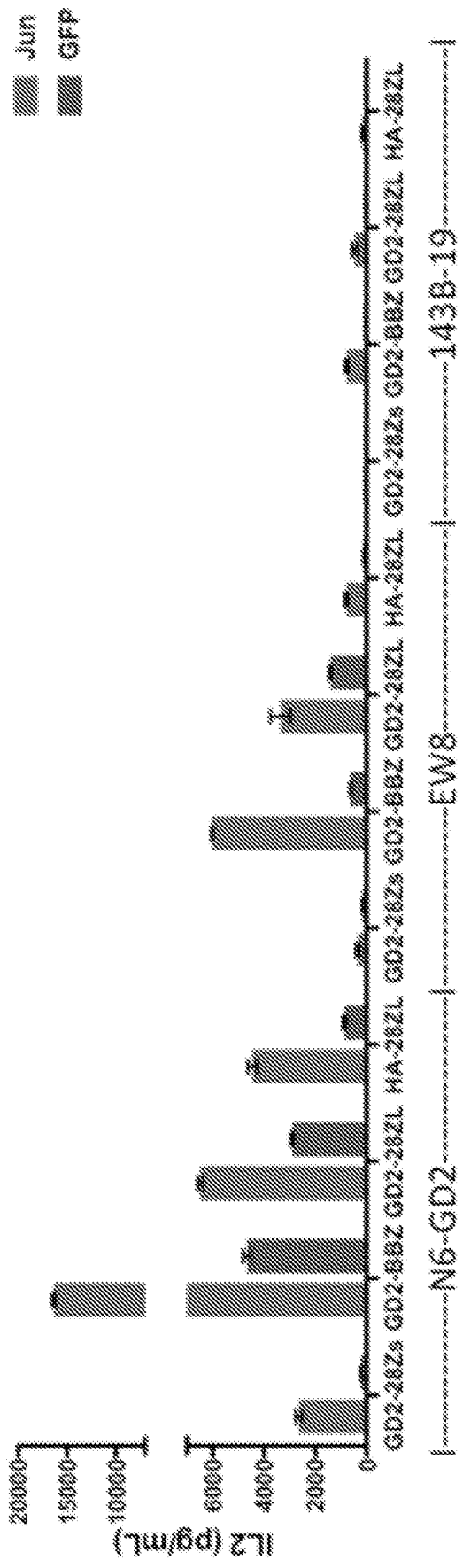

CD4 IL2
%IL2+
CD4 IFNg
% IFNg+
Jun
GFP
CD8 IL2
%IL2+
CD8 IFNg
% IFNg+
Jun
GFP

FIG. 4D


IL2 ELISA GD2
Jun
GFP
IL2 (pg/mL)
20000
15000
10000
6000
4000
2000
0
GD2-28Zs
GD2-BBZ
GD2-28ZL
HA-28ZL
GD2-28Zs
GD2-BBZ
HA-28ZL
GD2-28Zs
GD2-BBZ
GD2-28ZL
HA-28ZL
N6-GD2
EW8
143B-19

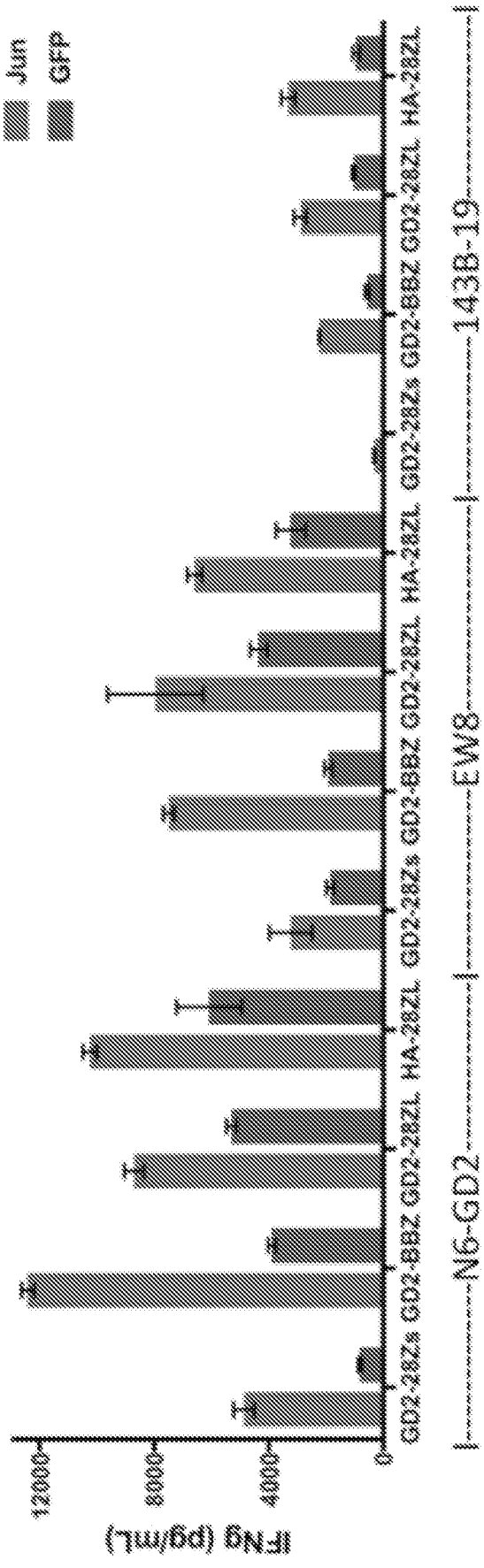
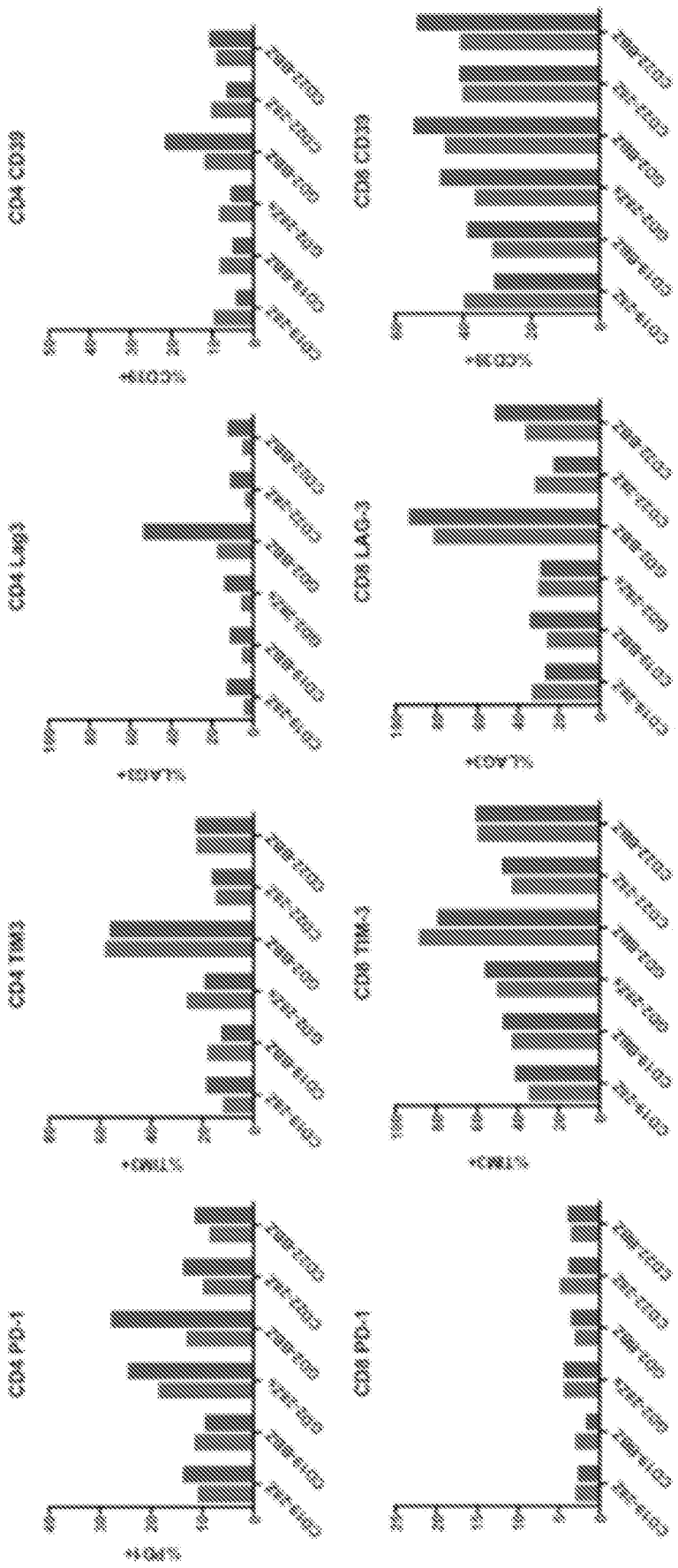

IFNg ELISA GD2
Jun
GFP
IFNg (pg/mL)
12000
8000
4000
0
GD2-28Zs
GD2-BBZ
GD2-28ZL
HA-28ZL
GD2-28Zs
GD2-BBZ
GD2-28ZL
HA-28ZL
GD2-28Zs
GD2-BBZ
GD2-28ZL
HA-28ZL
N6-GD2
EW8
143B-19

FIG. 4E

FIG. 5A


CD4 CD39
CD8 CD39
CD4 LAG3
CD8 LAG-3
CD4 TIM3
CD8 TIM-3
CD4 PD-1
CD8 PD-1

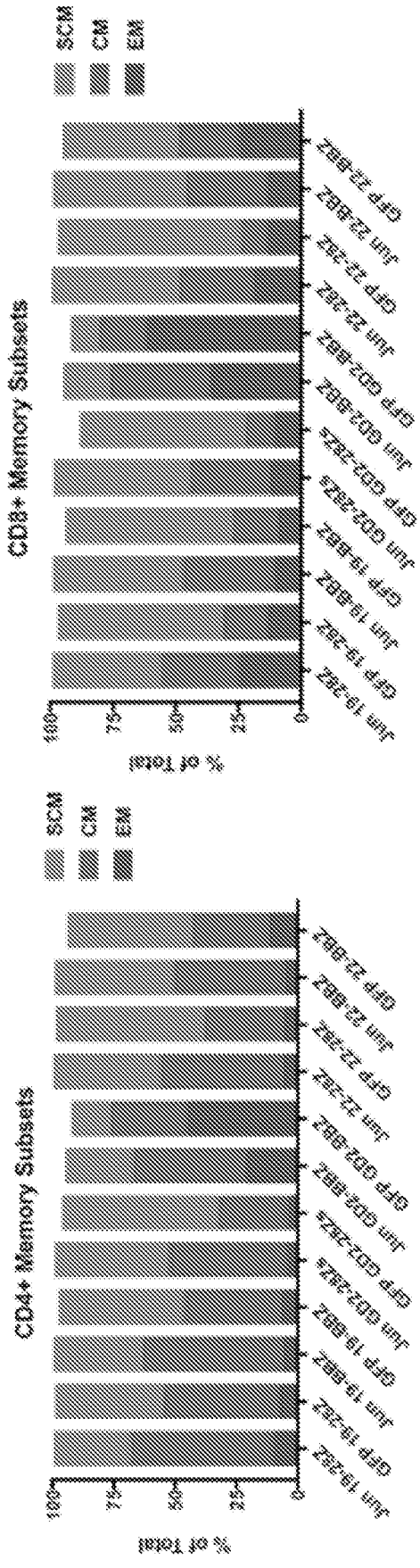

CD8+ Memory Subsets
SCM
CM
EM
% of Total
100
75
50
25
0
19-28z unt
19-28z GFP
19-BBz unt
19-BBz GFP
CD22-28z unt
CD22-28z GFP
CD22-BBz unt
CD22-BBz GFP
22-28z unt
22-28z GFP
22-BBz unt
22-BBz GFP
CD4+ Memory Subsets
SCM
CM
EM
% of Total
100
75
50
25
0
19-28z unt
19-28z GFP
19-BBz unt
19-BBz GFP
CD22-28z unt
CD22-28z GFP
CD22-BBz unt
CD22-BBz GFP
22-28z unt
22-28z GFP
22-BBz unt
22-BBz GFP

FIG. 5B

FIG. 5C

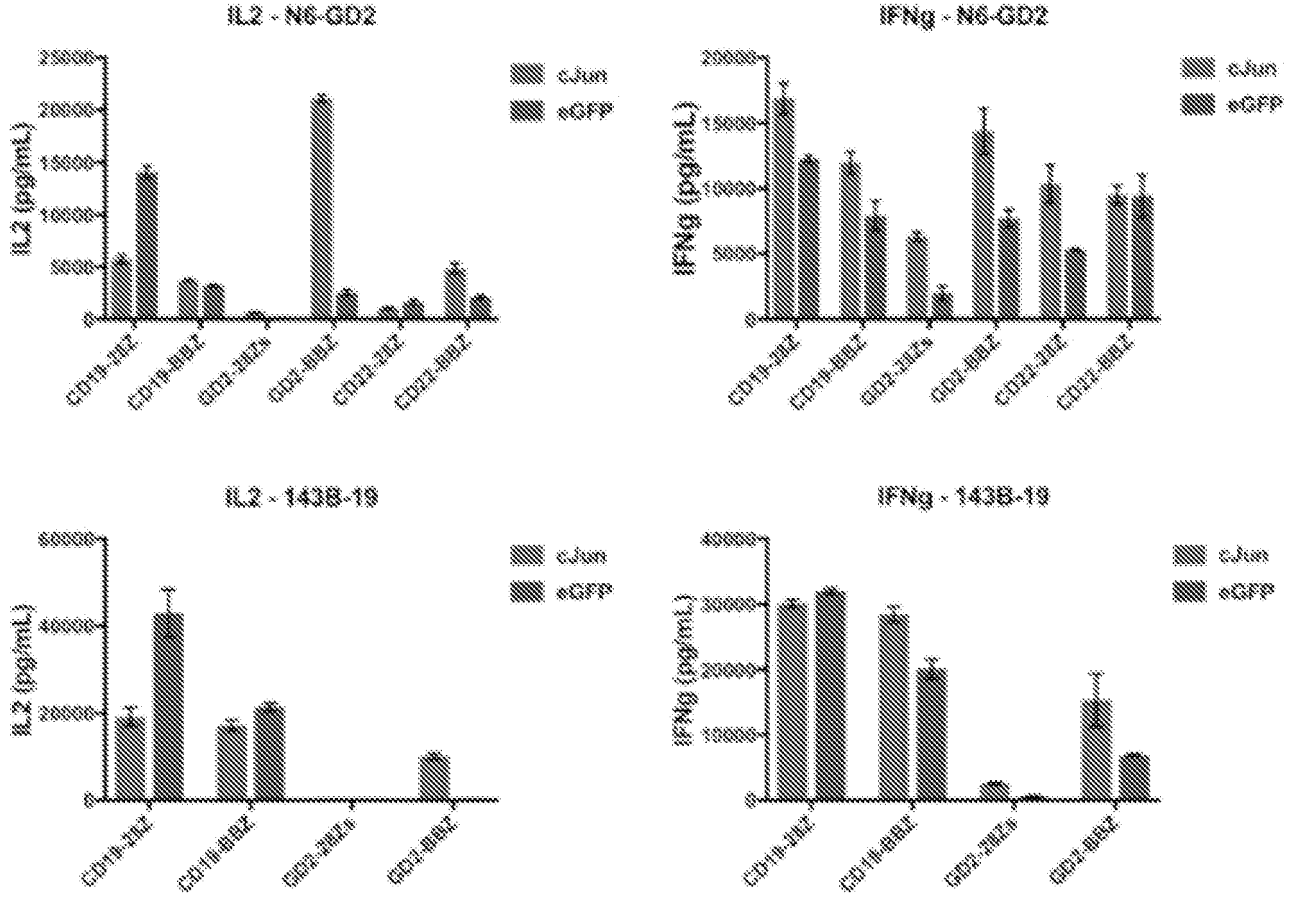

IL2 - N6-GD2
IFNg - N6-GD2
IL2 - 143B-19
IFNg - 143B-19
cJun
eGFP
IL2 (pg/mL)
IFNg (pg/mL)

FIG. 6A

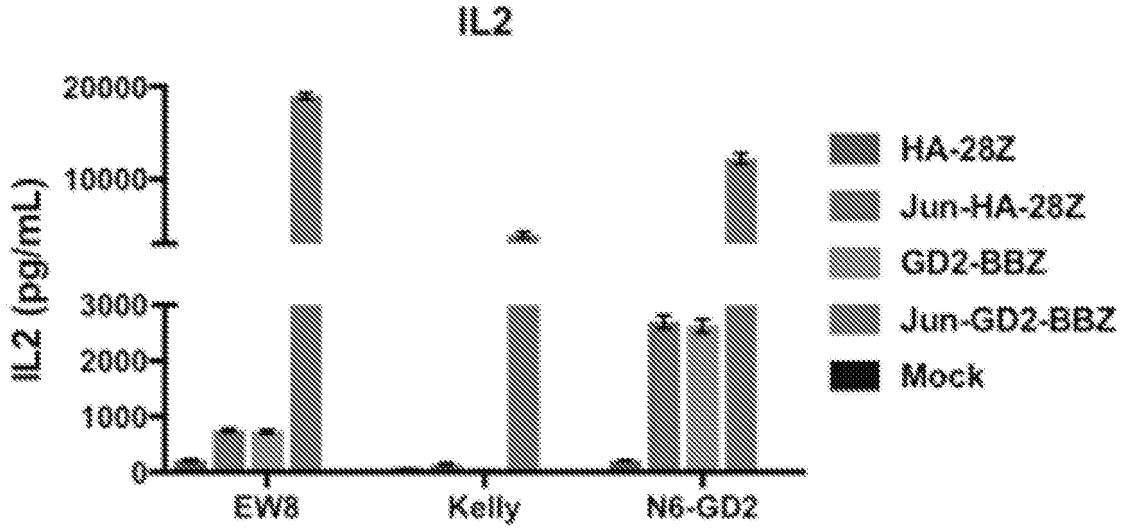

IL2
IL2 (pg/mL)
20000
10000
3000
2000
1000
0
EW8
Kelly
N6-GD2
HA-28Z
Jun-HA-28Z
GD2-BBZ
Jun-GD2-BBZ
Mock

FIG. 6B

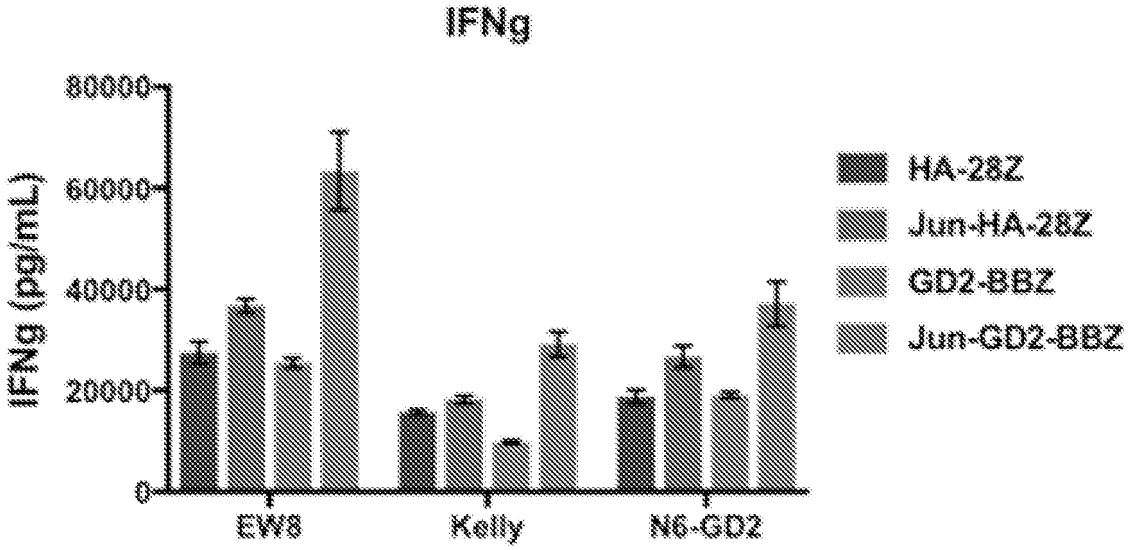

IFNg
IFNg (pg/mL)
80000
60000
40000
20000
0
EW8
Kelly
N6-GD2
HA-28Z
Jun-HA-28Z
GD2-BBZ
Jun-GD2-BBZ

FIG. 6C

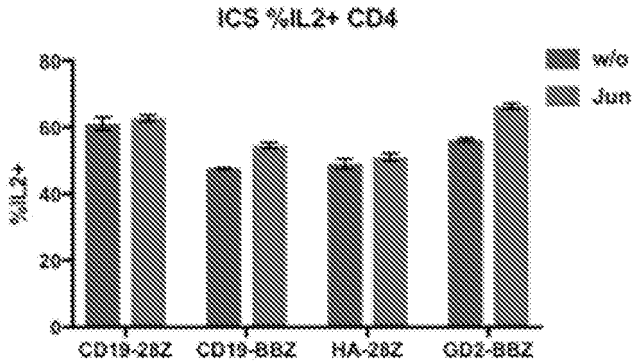
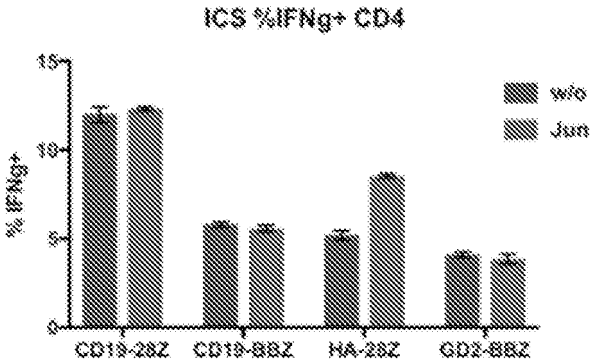
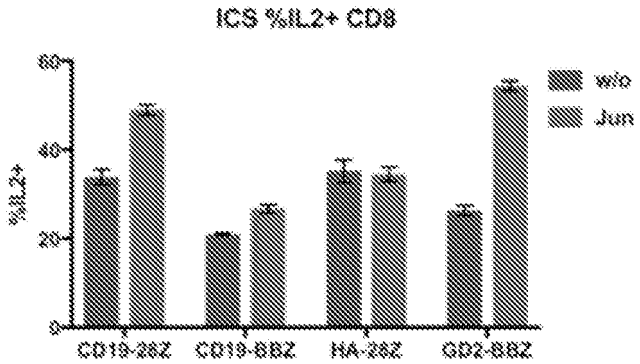


FIG. 6D





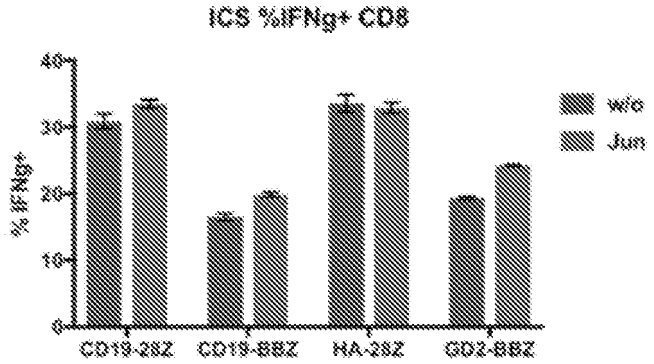


FIG. 6E

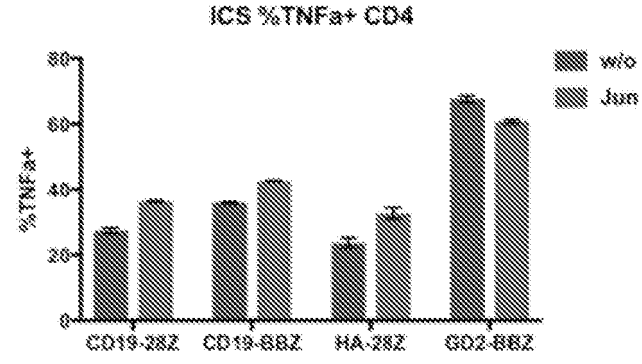
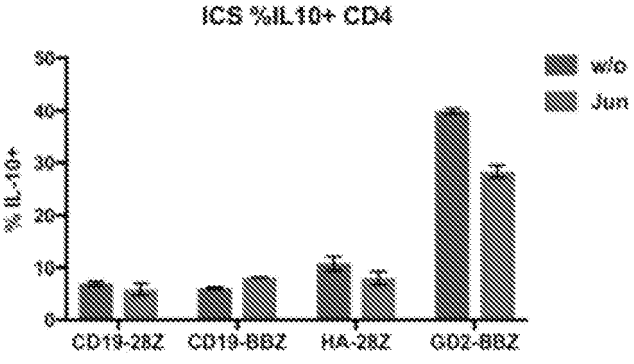
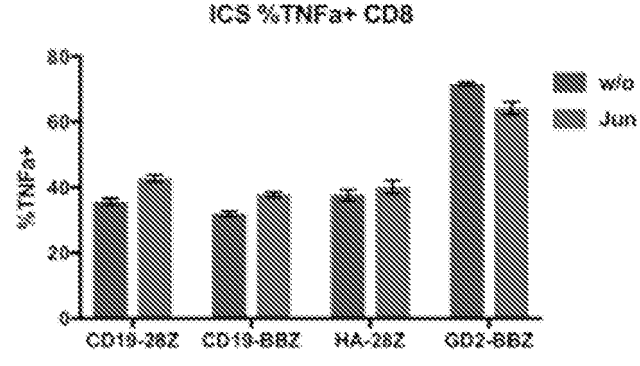


FIG. 6F





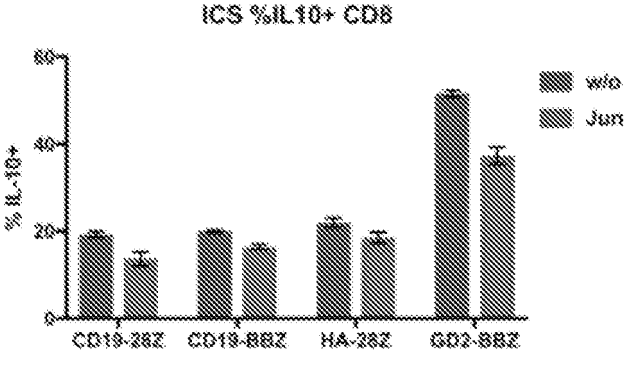


FIG. 7A

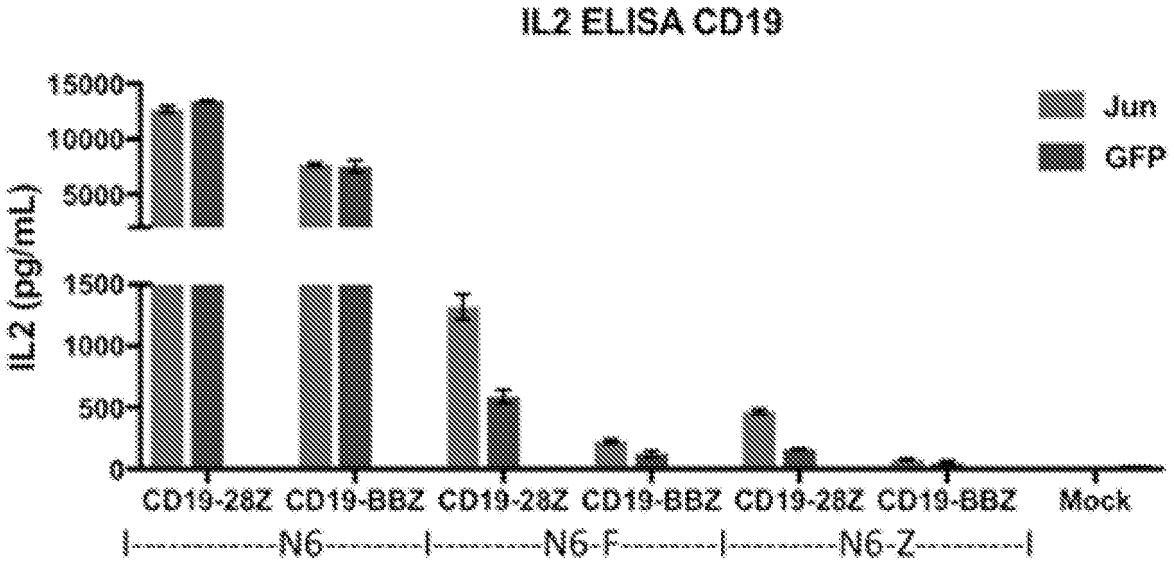

IL2 ELISA CD19
IL2 (pg/mL)
15000
10000
5000
1500
1000
500
0
Jun
GFP
CD19-28Z
CD19-BBZ
CD19-28Z
CD19-BBZ
CD19-28Z
CD19-BBZ
Mock
N6
N6-F
N6-Z

FIG. 7B

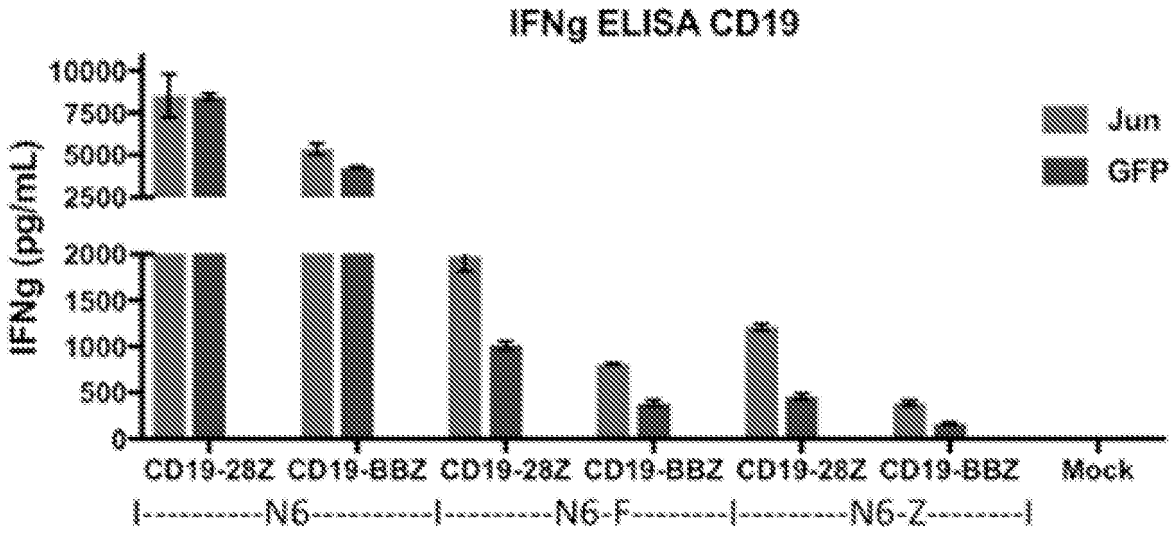

IFNg ELISA CD19
IFNg (pg/mL)
10000
7500
5000
2500
2000
1500
1000
500
0
Jun
GFP
CD19-28Z
CD19-BBZ
CD19-28Z
CD19-BBZ
CD19-28Z
CD19-BBZ
Mock
N6
N6-F
N6-Z

FIG. 7C

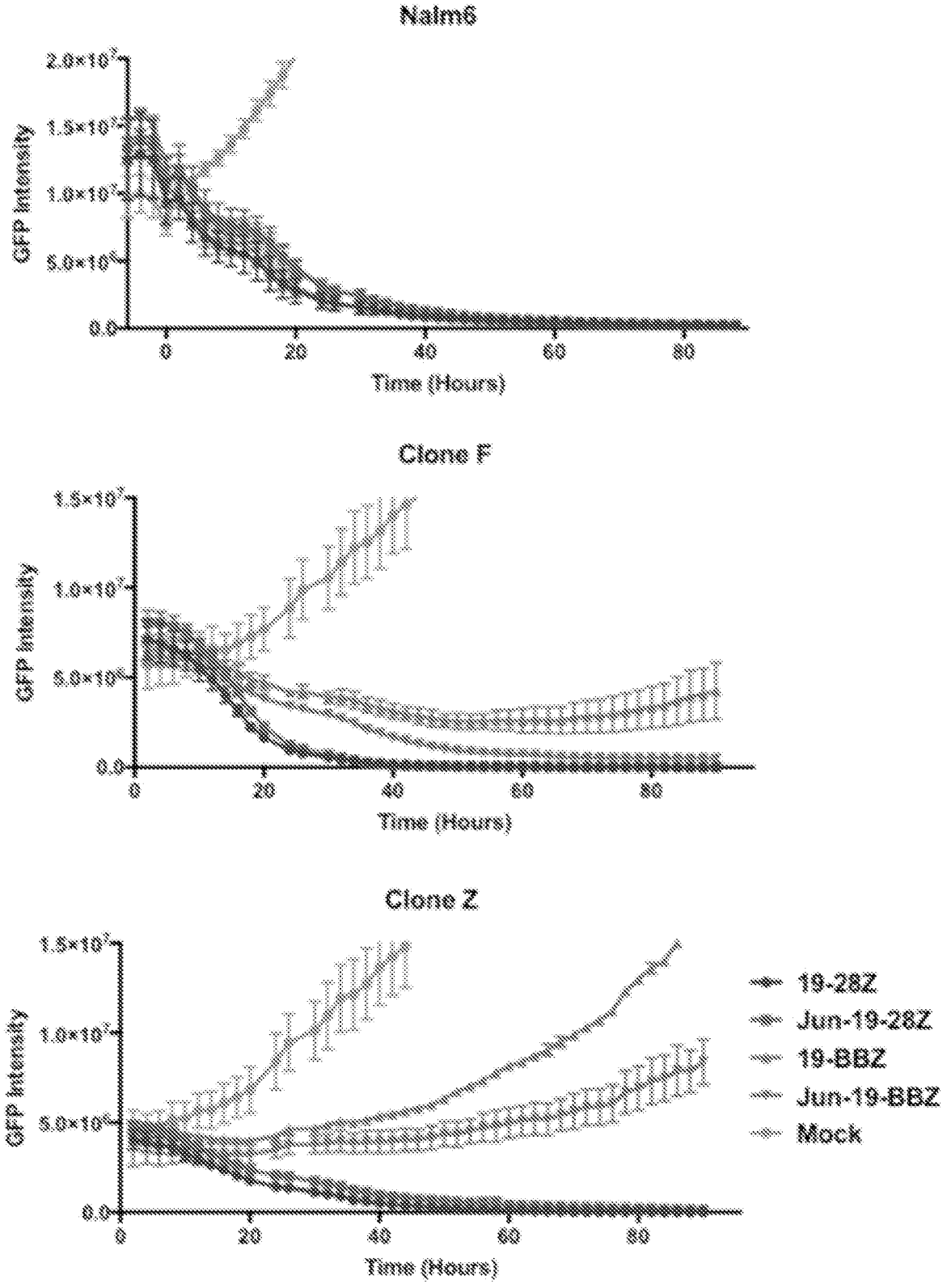

Nalm6
GFP Intensity
Time (Hours)
Clone F
GFP Intensity
Time (Hours)
Clone Z
GFP Intensity
Time (Hours)
19-28Z
Jun-19-28Z
19-BBZ
Jun-19-BBZ
Mock

FIG. 7D

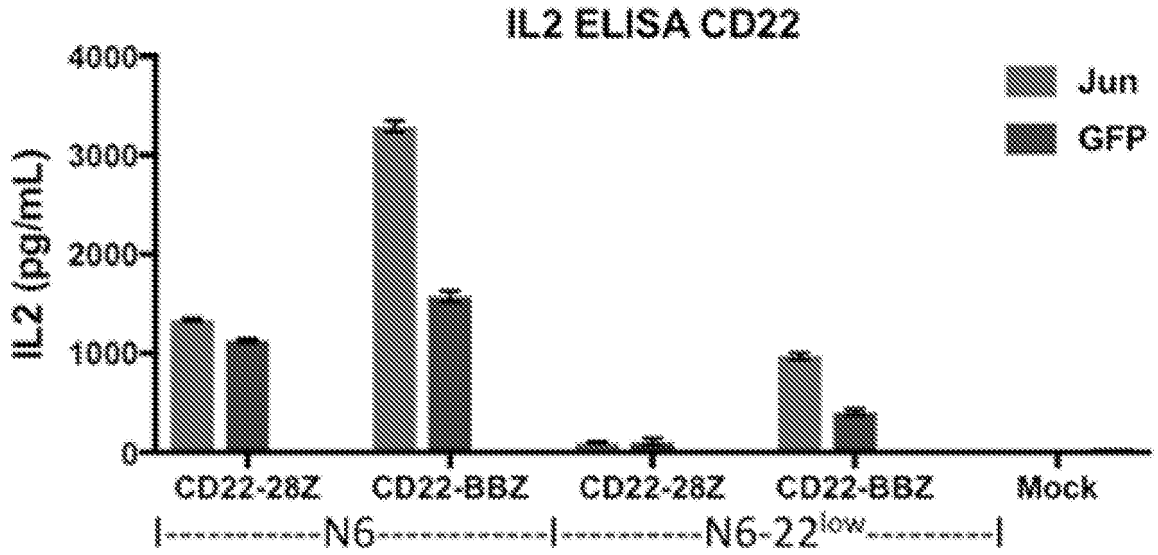

IL2 ELISA CD22
IL2 (pg/mL)
4000
3000
2000
1000
0
Jun
GFP
CD22-28Z
CD22-BBZ
CD22-28Z
CD22-BBZ
Mock
N6
N6-22low

FIG. 7E

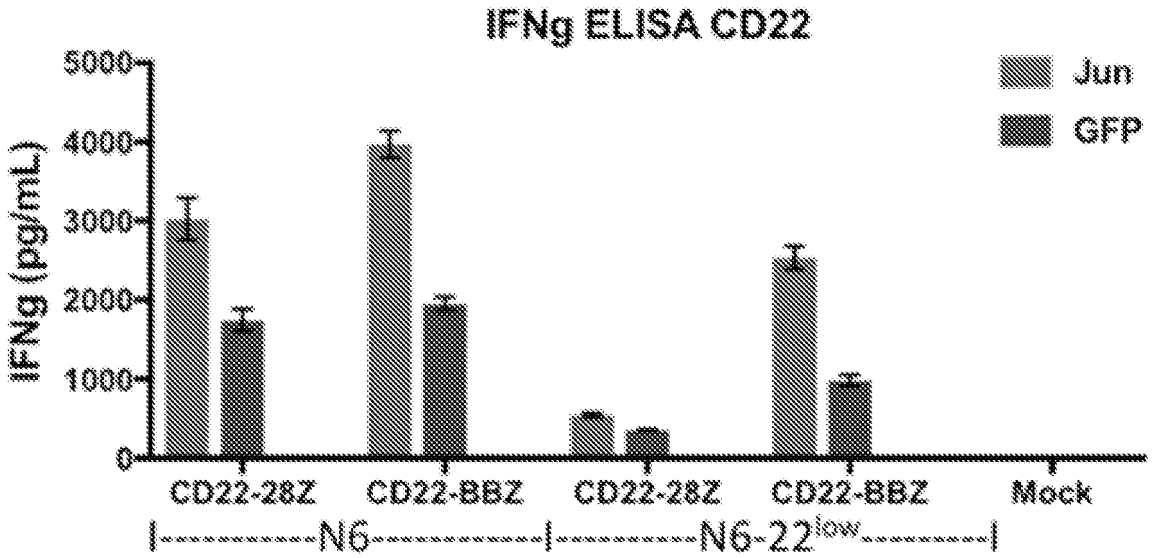

IFNg ELISA CD22
IFNg (pg/mL)
5000
4000
3000
2000
1000
0
Jun
GFP
CD22-28Z
CD22-BBZ
CD22-28Z
CD22-BBZ
Mock
N6
N6-22low

FIG. 8A

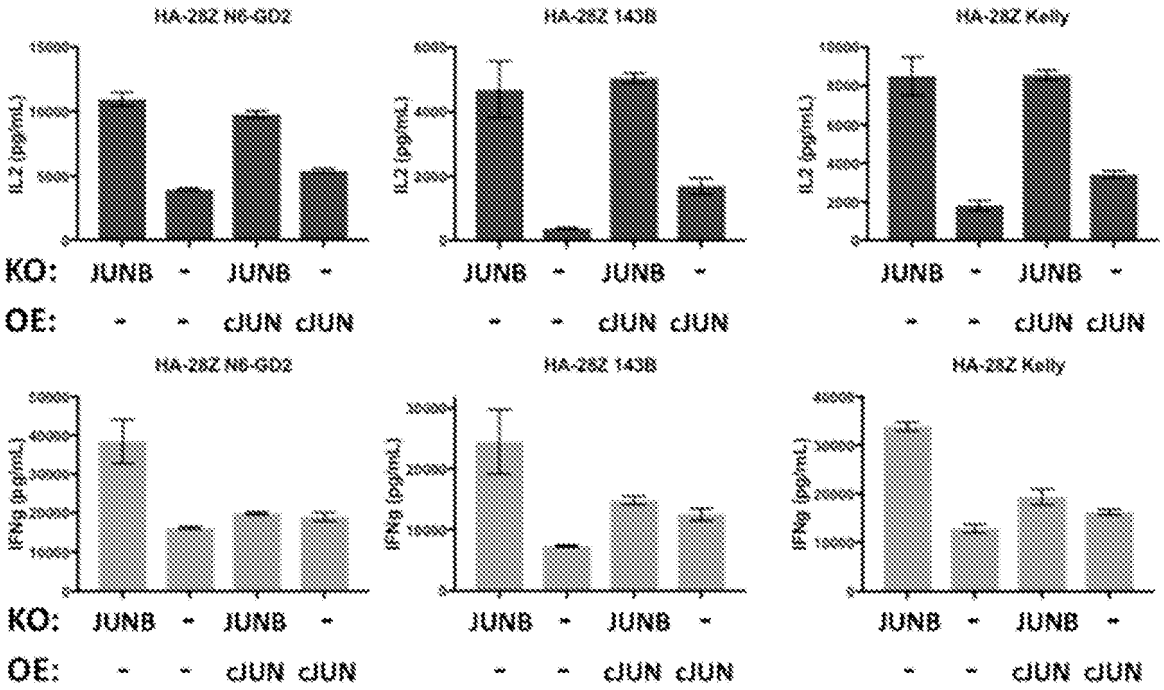

HA-28Z N6-GD2
HA-28Z 143B
HA-28Z Kelly
IL2 (pg/mL)
IFNg (pg/mL)
KO: JUNB - JUNB -
OE: - - cJUN cJUN

FIG. 8B

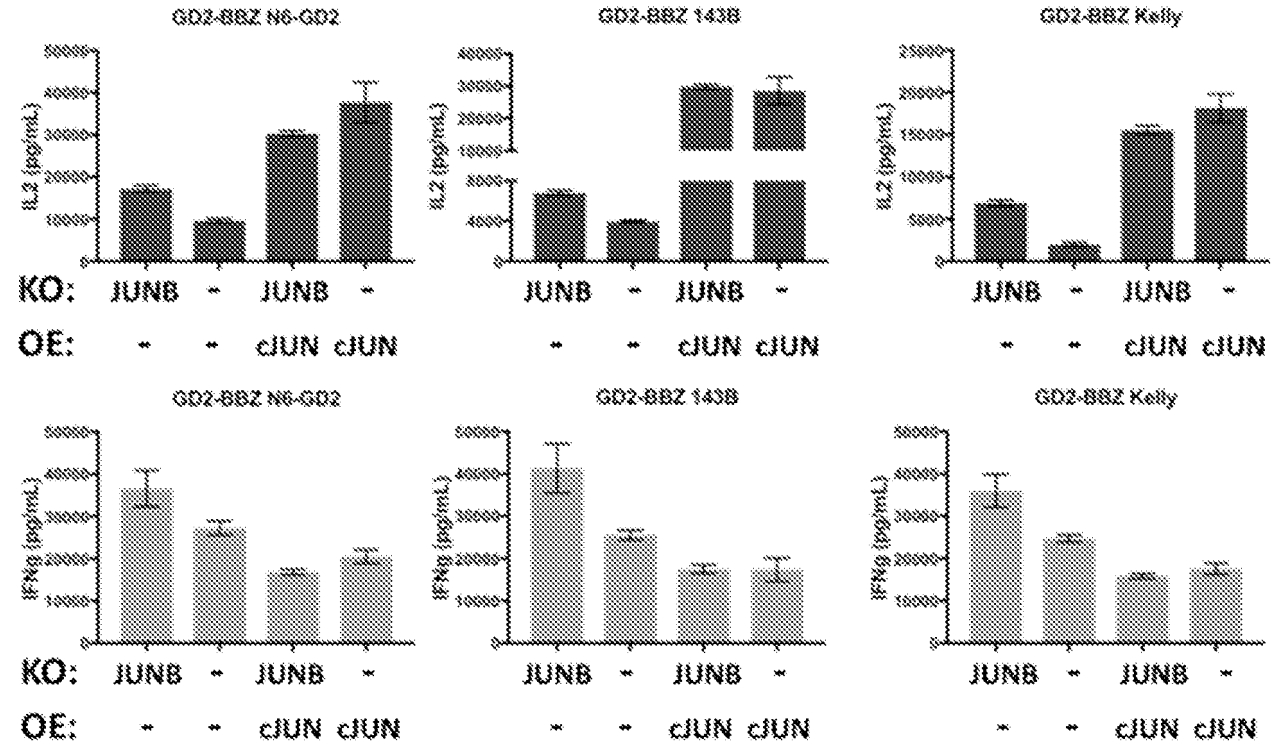

GD2-BBZ N6-GD2
GD2-BBZ 143B
GD2-BBZ Kelly
IL2 (pg/mL)
IFNg (pg/mL)
KO: JUNB - JUNB -
OE: - - cJUN cJUN

FIG. 8C

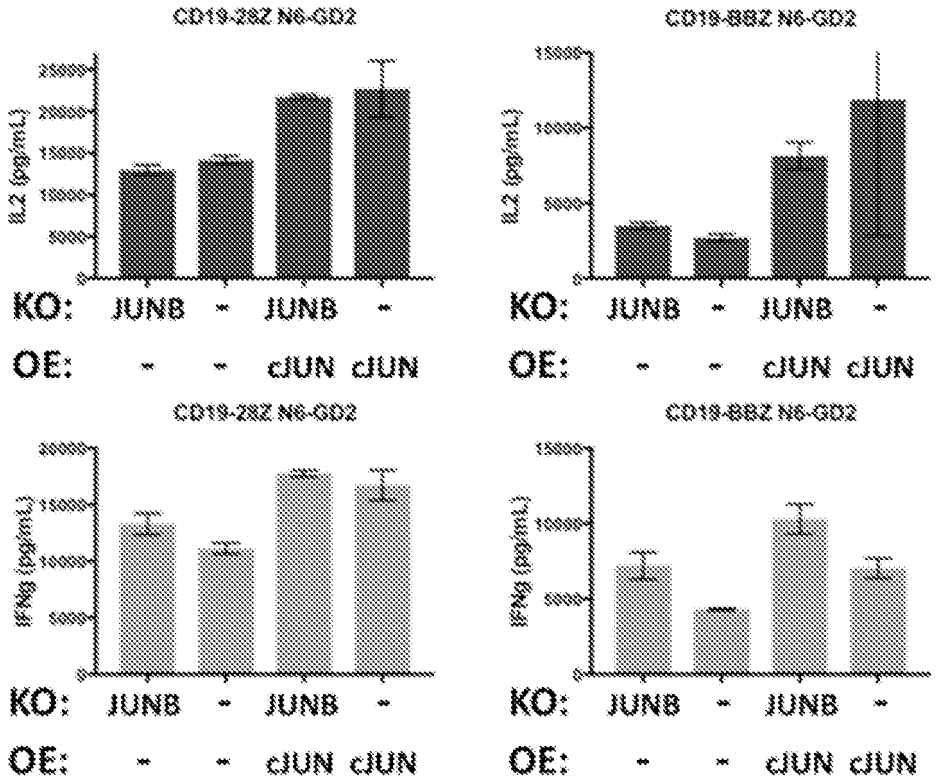

CD19-28Z N6-GD2
IL2 (pg/mL)
KO: JUNB - JUNB -
OE: - - cJUN cJUN
CD19-BBZ N6-GD2
IL2 (pg/mL)
KO: JUNB - JUNB -
OE: - - cJUN cJUN
CD19-28Z N6-GD2
IFNg (pg/mL)
KO: JUNB - JUNB -
OE: - - cJUN cJUN
CD19-BBZ N6-GD2
IFNg (pg/mL)
KO: JUNB - JUNB -
OE: - - cJUN cJUN

FIG. 8D

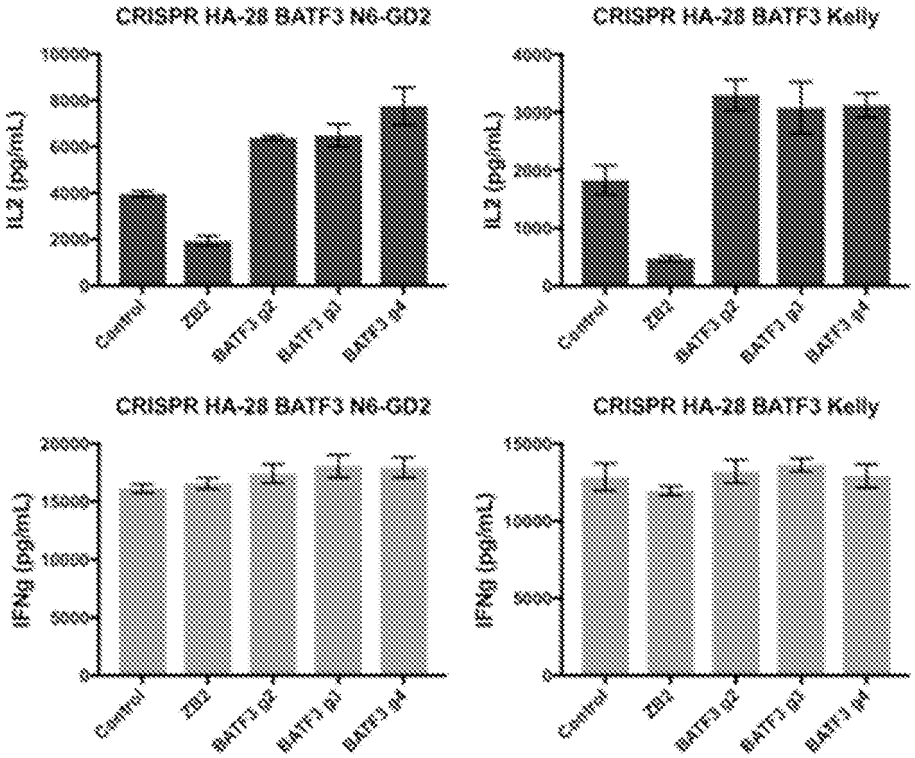

CRISPR HA-28 BATF3 N6-GD2
IL2 (pg/mL)
CRISPR HA-28 BATF3 Kelly
IL2 (pg/mL)
CRISPR HA-28 BATF3 N6-GD2
IFNg (pg/mL)
CRISPR HA-28 BATF3 Kelly
IFNg (pg/mL)

FIG. 9A

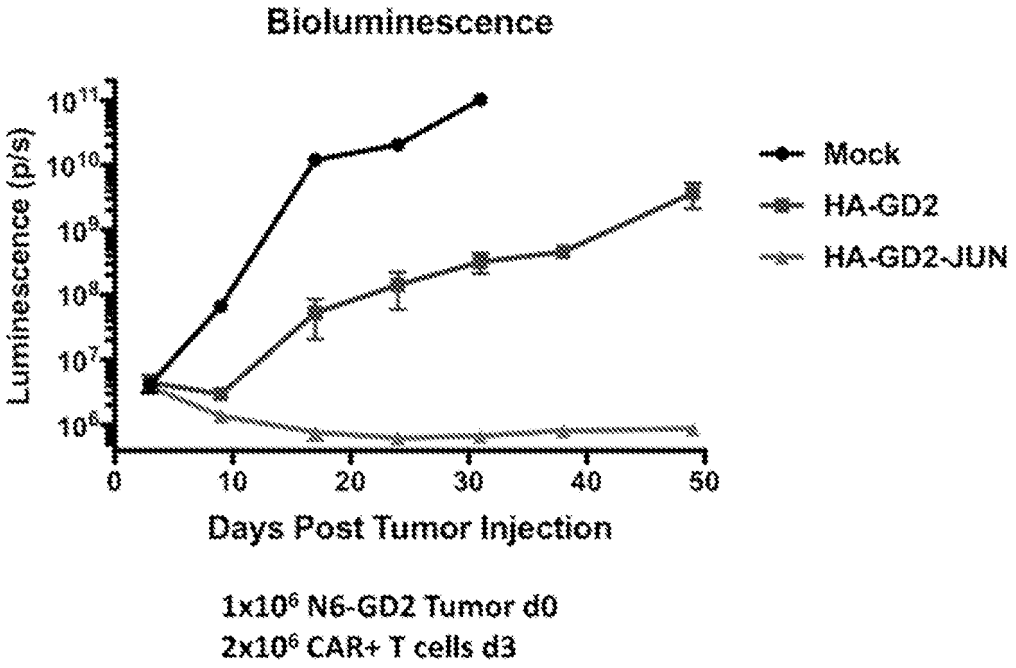

Bioluminescence
Luminescence (p/s)
Days Post Tumor Injection
Mock
HA-GD2
HA-GD2-JUN
1x10⁶ N6-GD2 Tumor d0
2x10⁶ CAR+ T cells d3

FIG. 9B

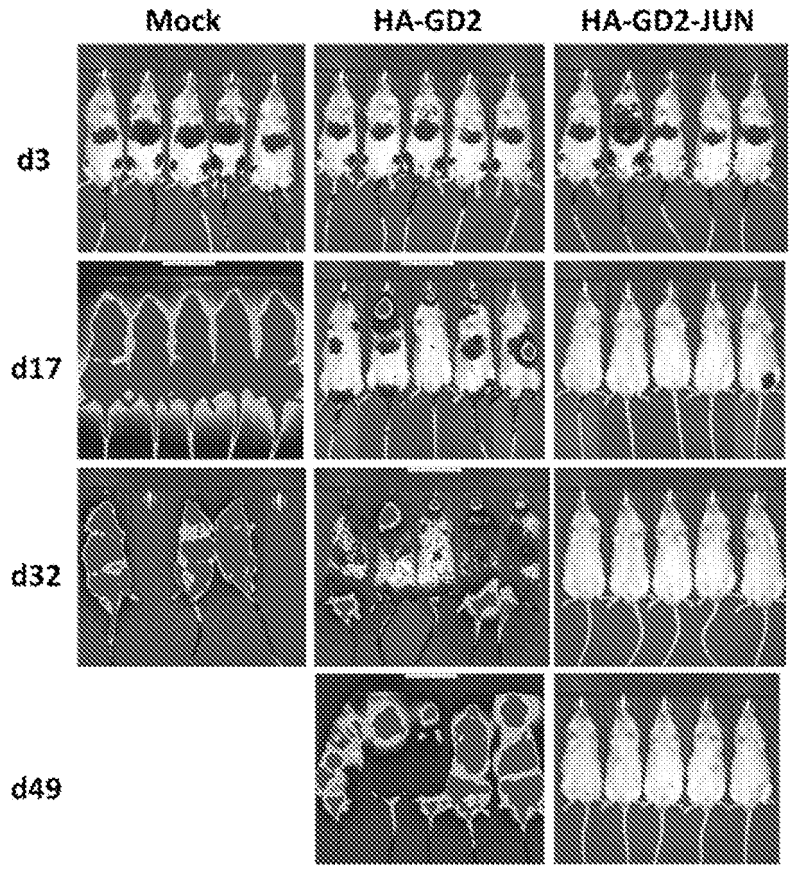

Mock
HA-GD2
HA-GD2-JUN
d3
d17
d32
d49

FIG. 10

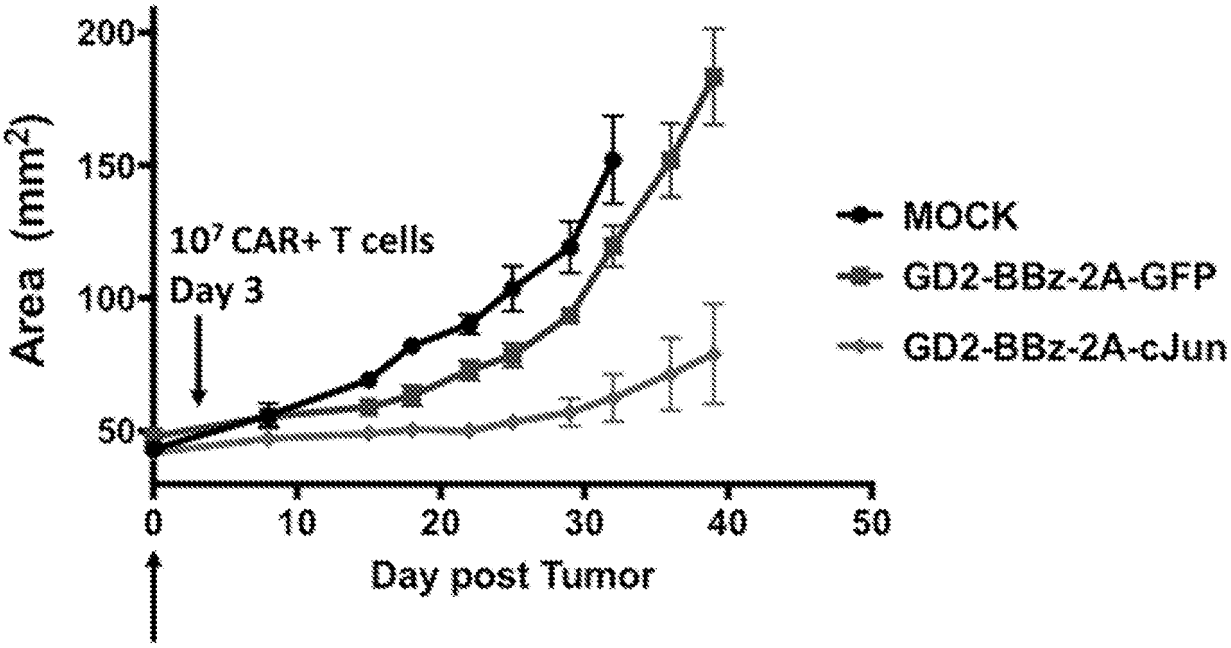

200
150
100
50
Area (mm2)
10^7 CAR+ T cells
Day 3
0
10
20
30
40
50
Day post Tumor
MOCK
GD2-BBz-2A-GFP
GD2-BBz-2A-cJun
5x10^5 143B Tumor Cells im
Day 0

FIG. 11A

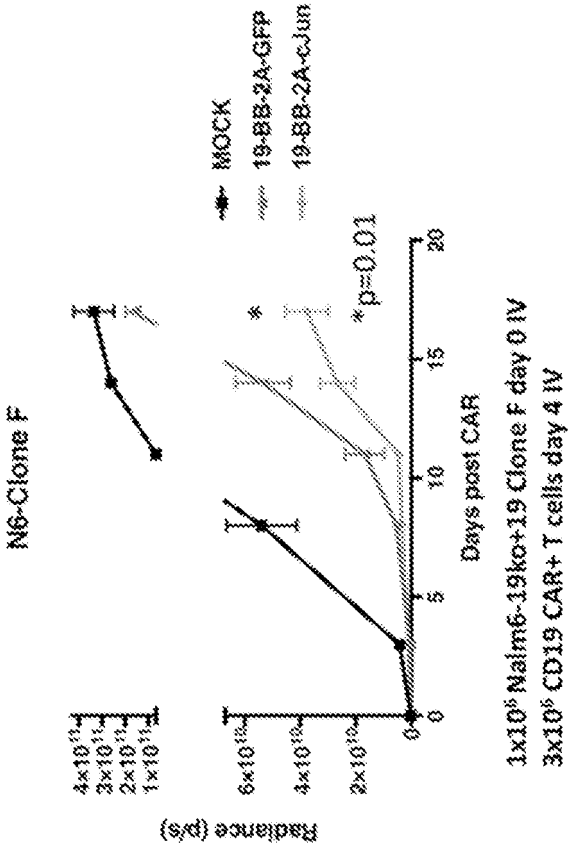


FIG. 11B

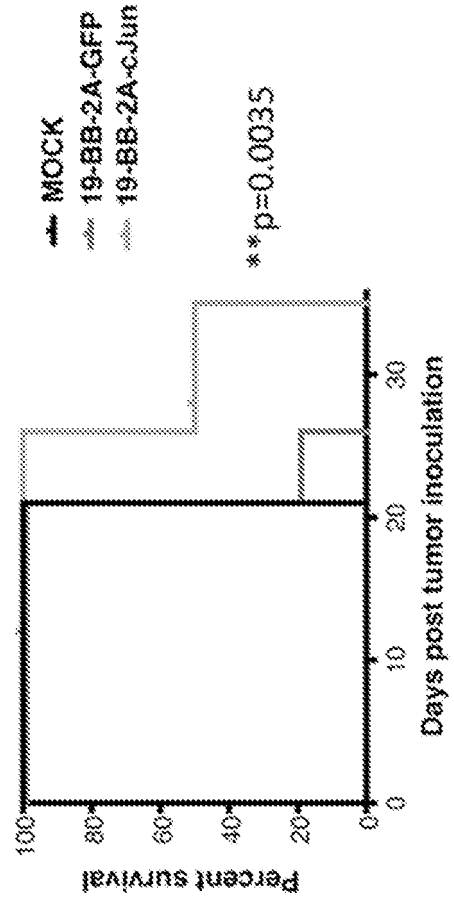


FIG. 11C

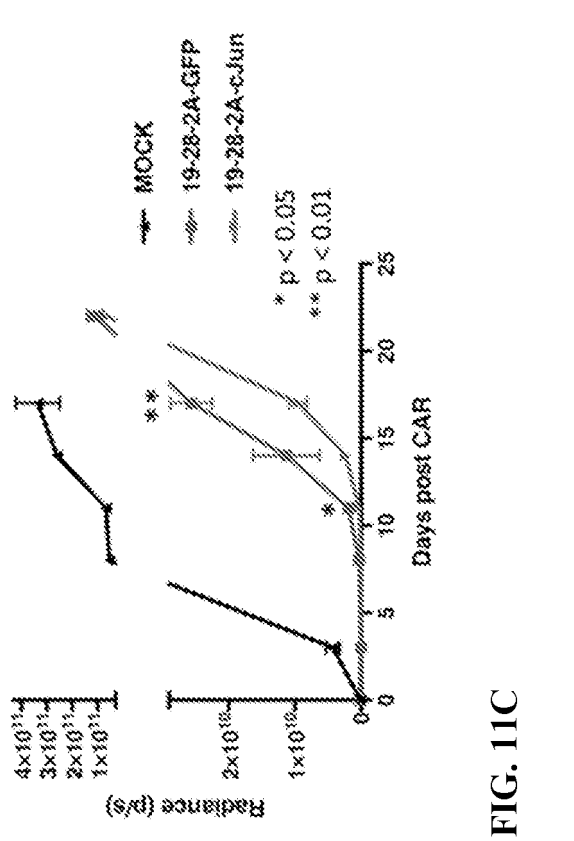


FIG. 11D

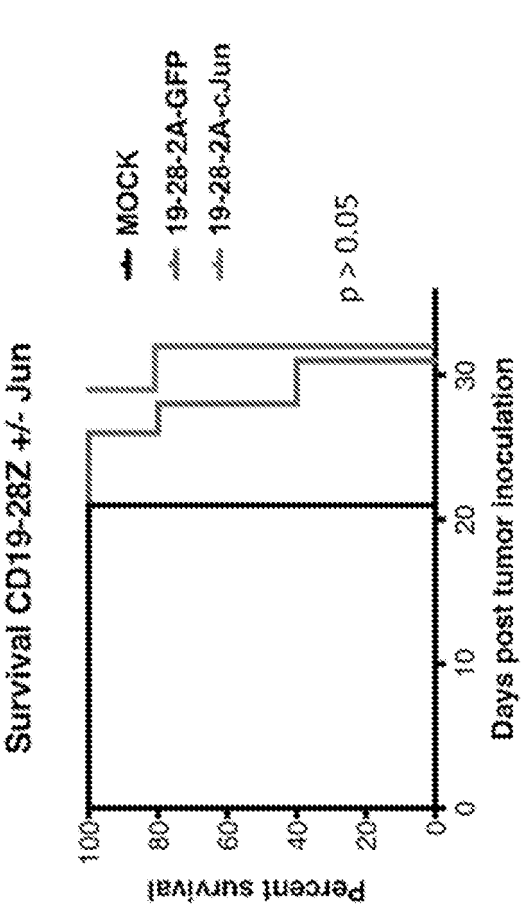


FIG. 12A

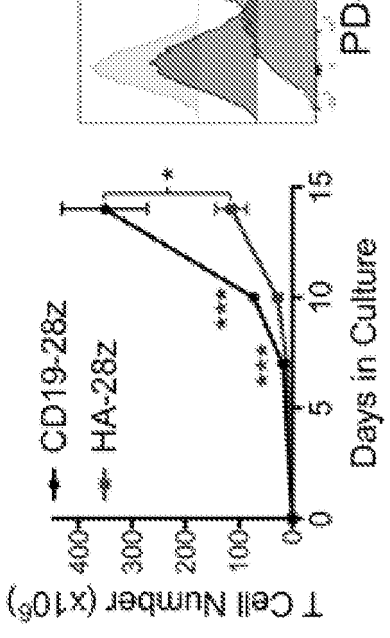


FIG. 12B

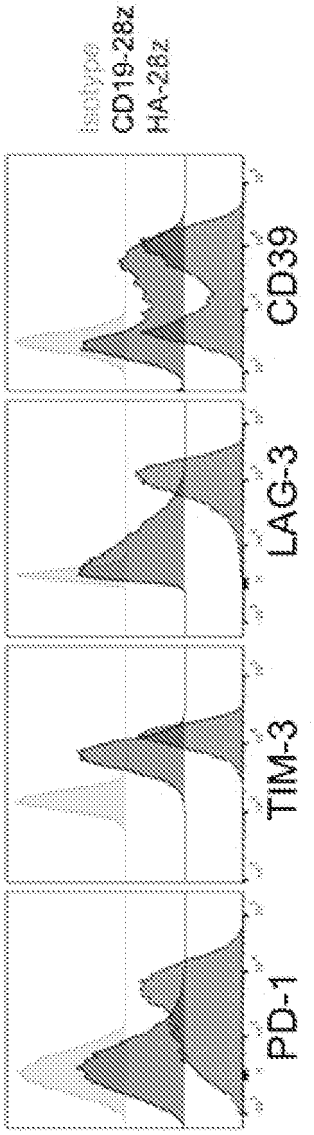


FIG. 12C

FIG. 12D

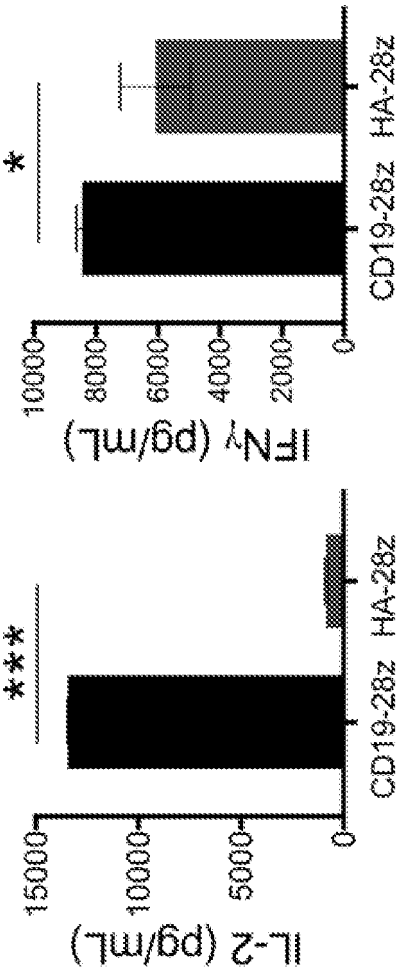


FIG. 12E

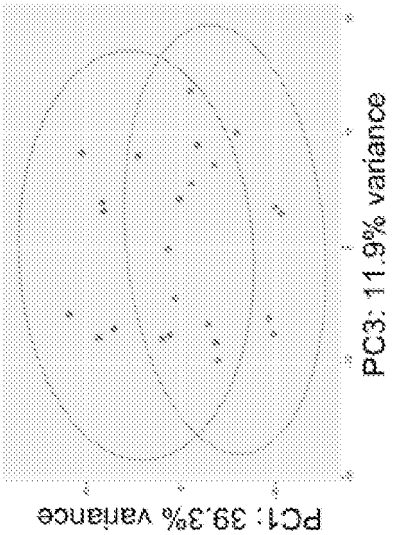


FIG. 12F

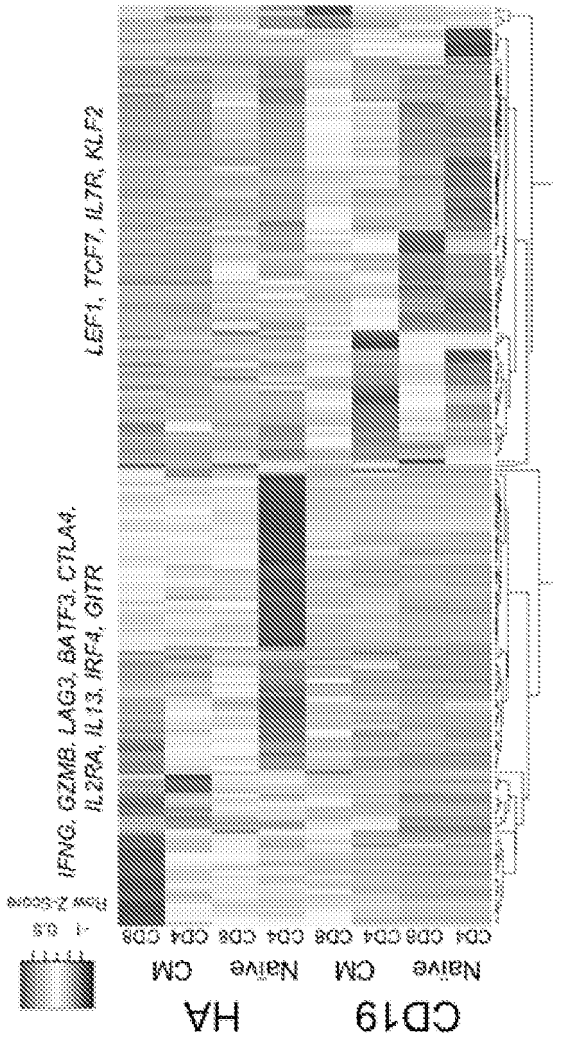


FIG. 12G

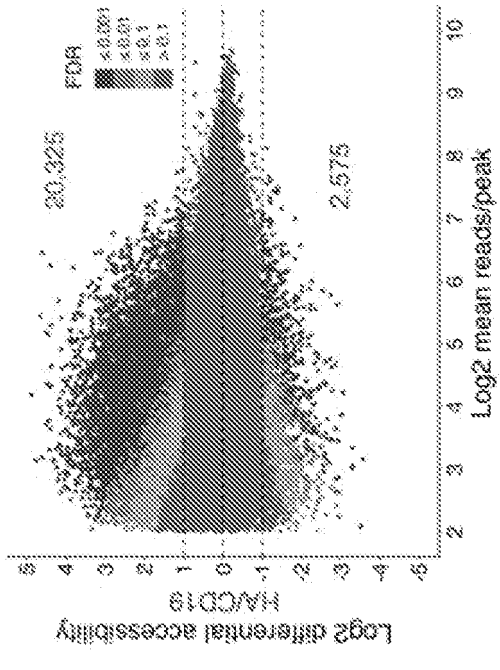


FIG. 12H

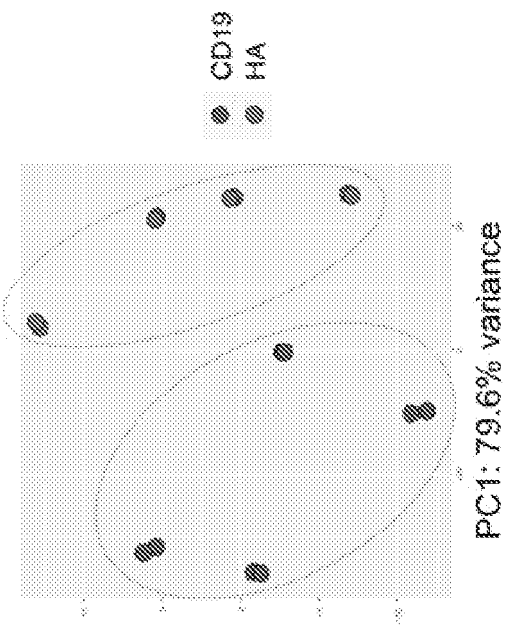


FIG. 12I

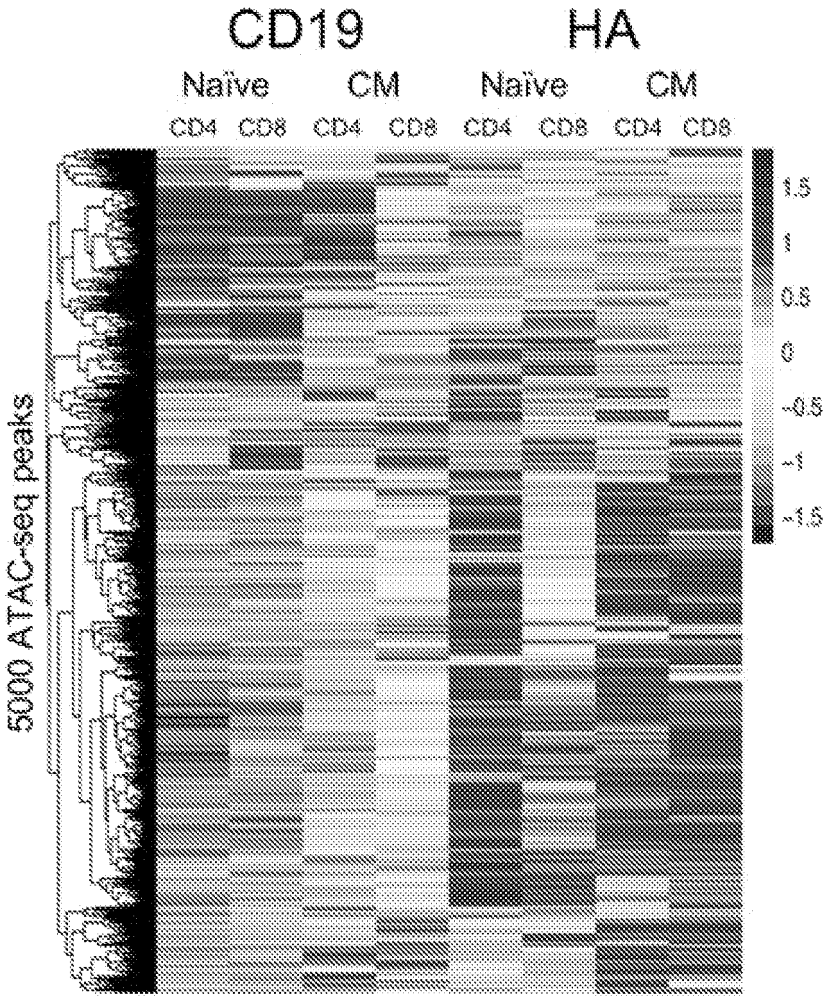


FIG. 12J

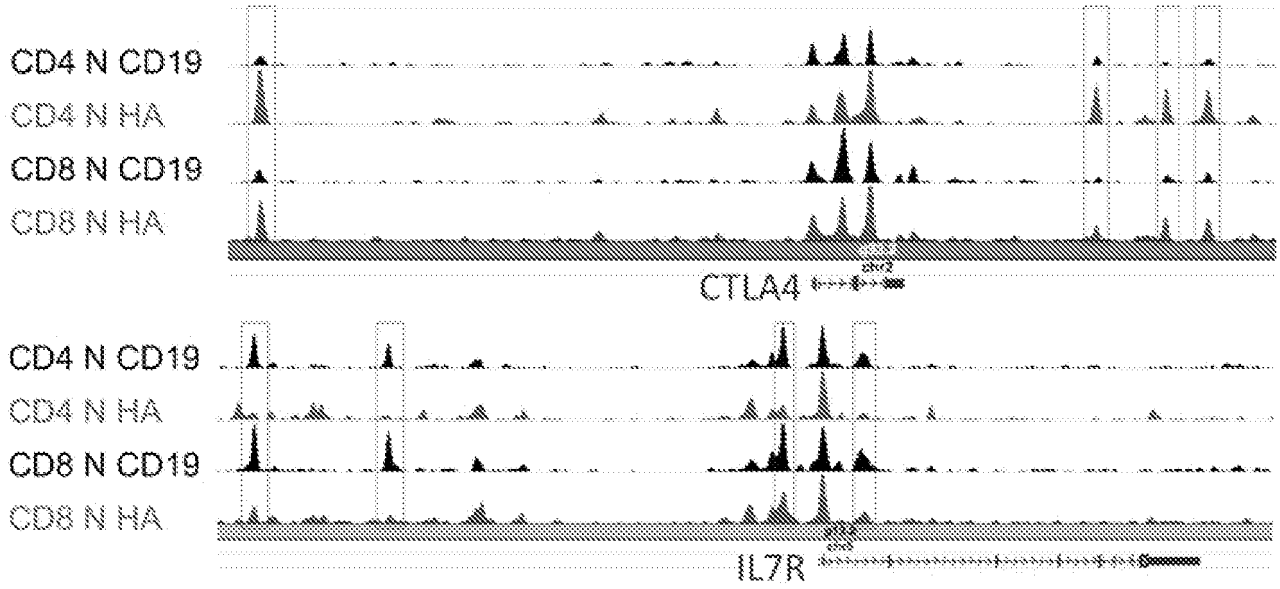


FIG. 13A

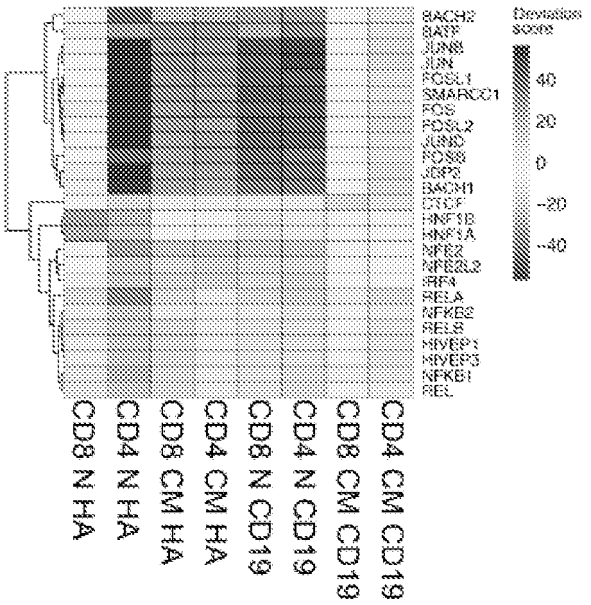


FIG. 13B

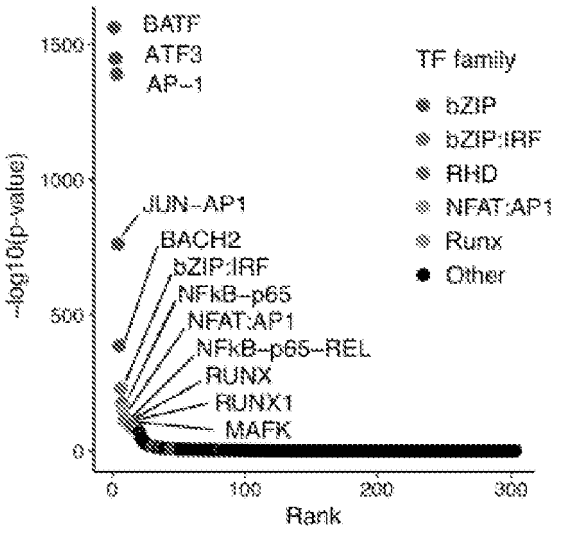


FIG. 13C

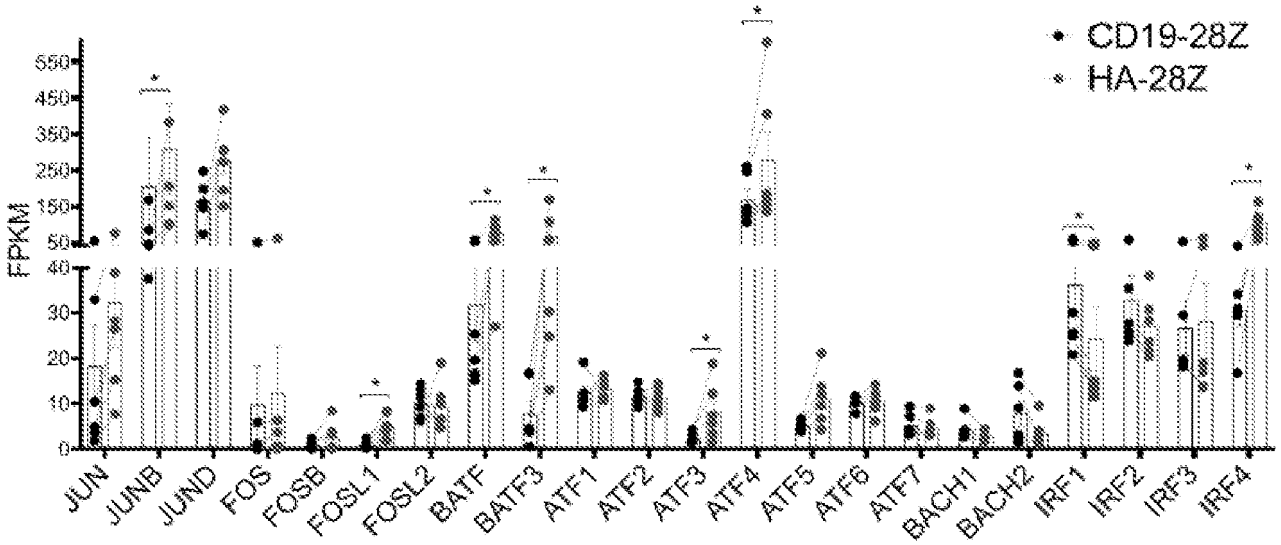


FIG. 13D

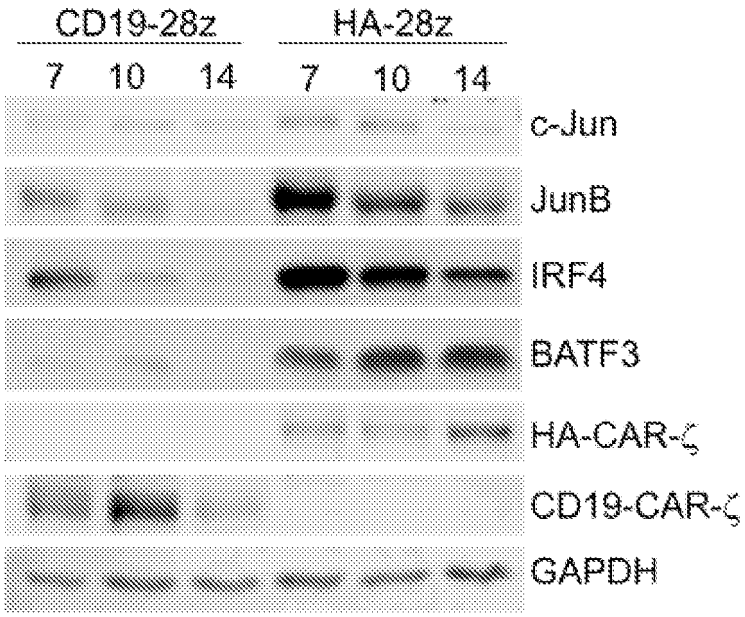


FIG. 13E

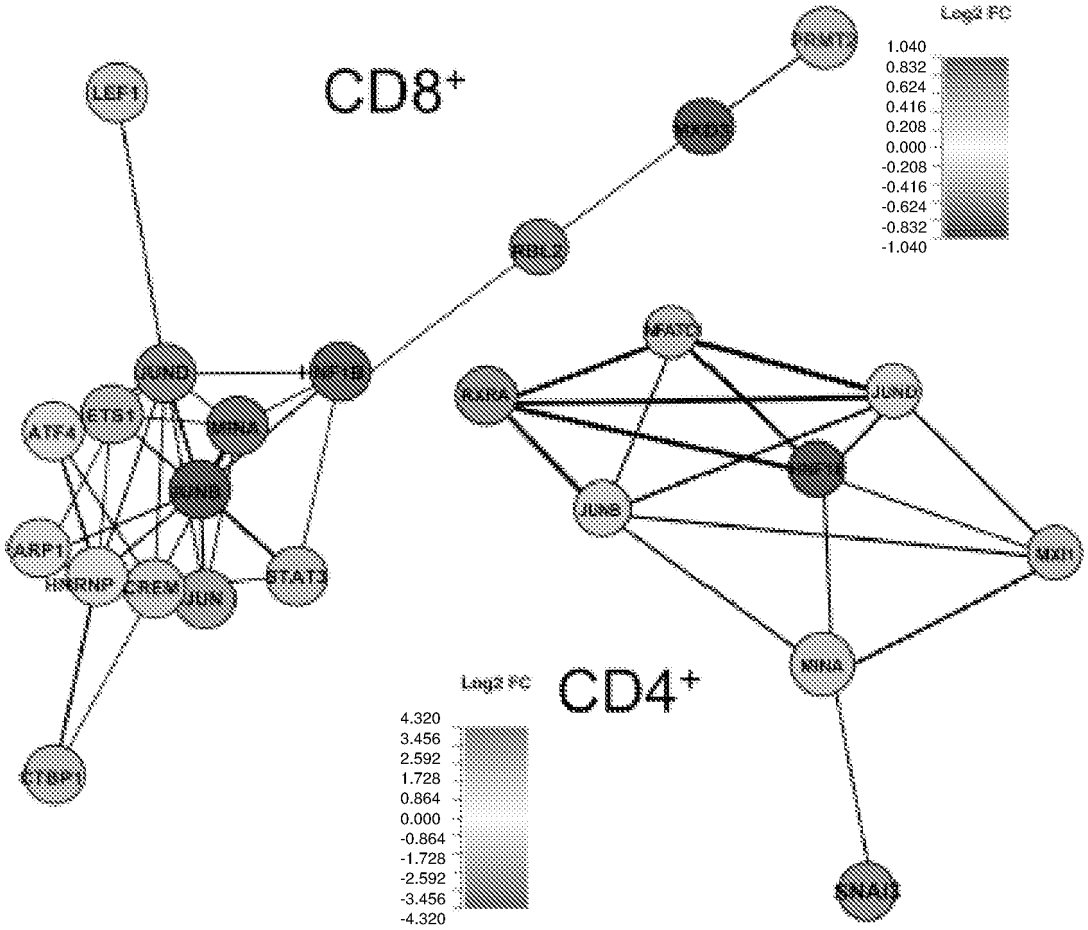


FIG. 14A

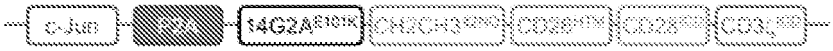


FIG. 14B

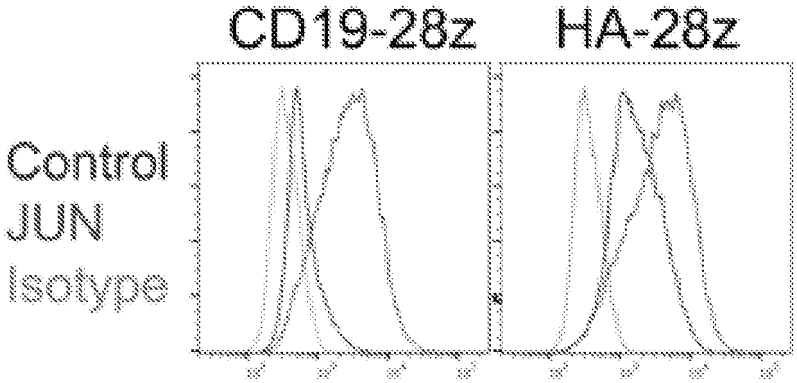


FIG. 14C

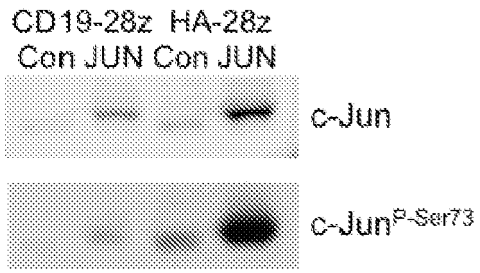


FIG. 14D

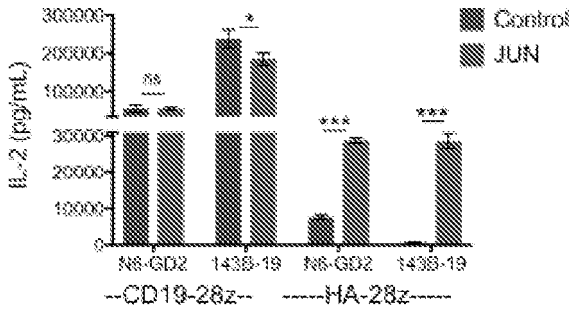


FIG. 14E

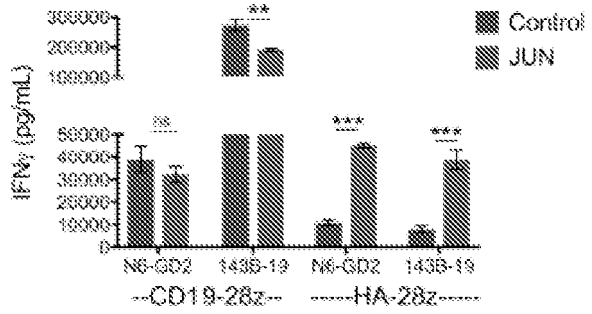


FIG. 14F

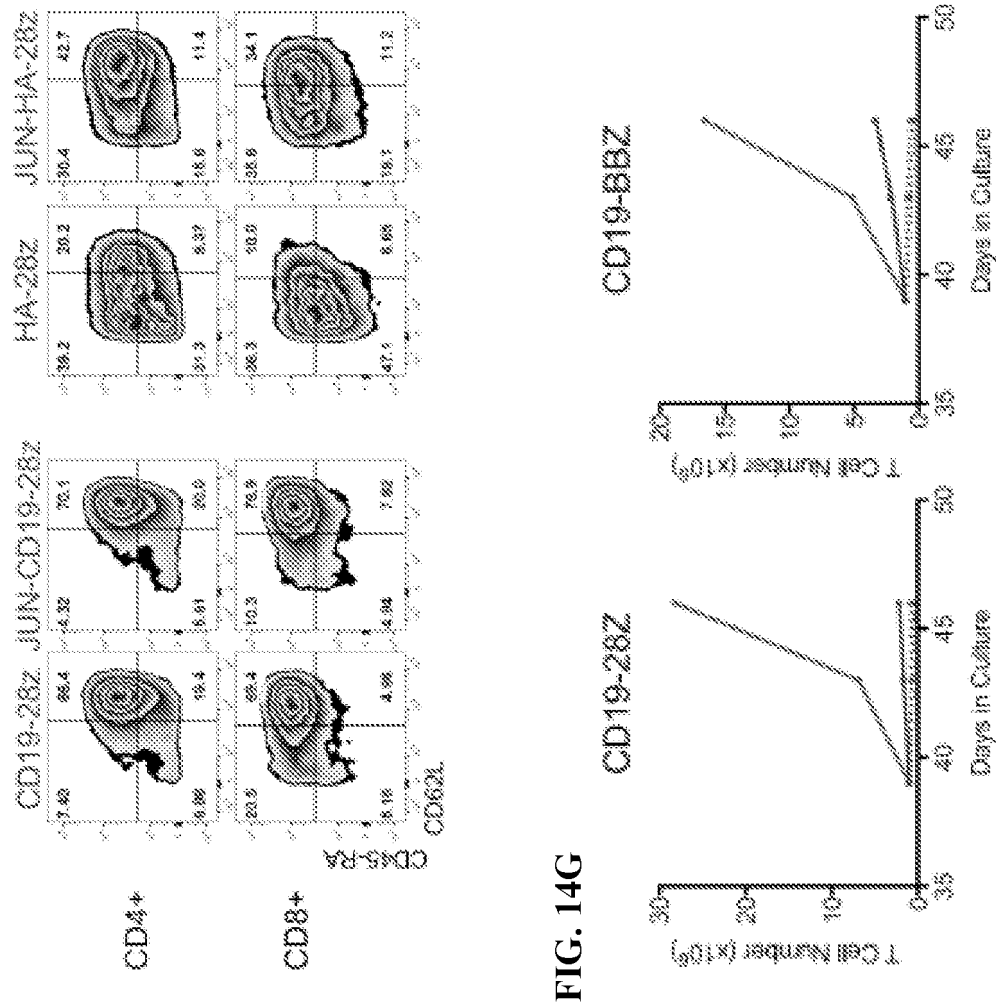


FIG. 14G

FIG. 14K

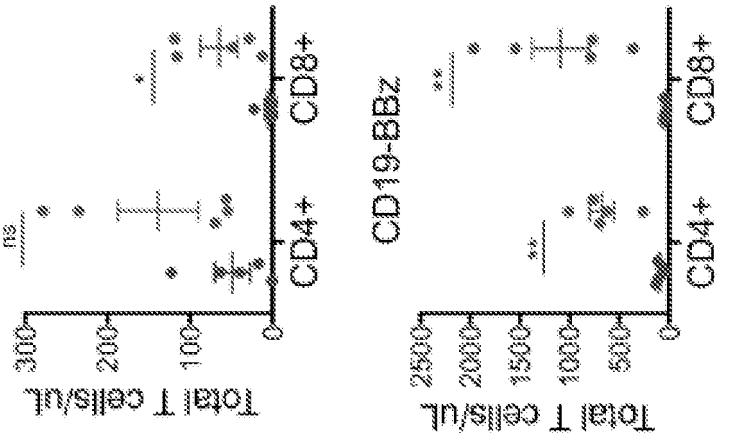


FIG. 14I

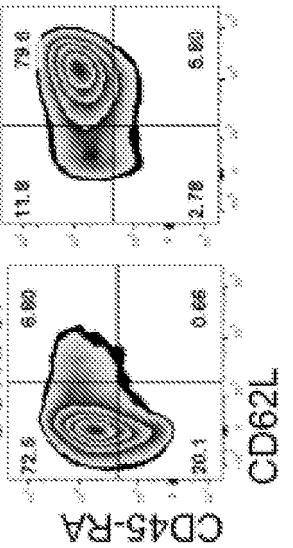


FIG. 14H

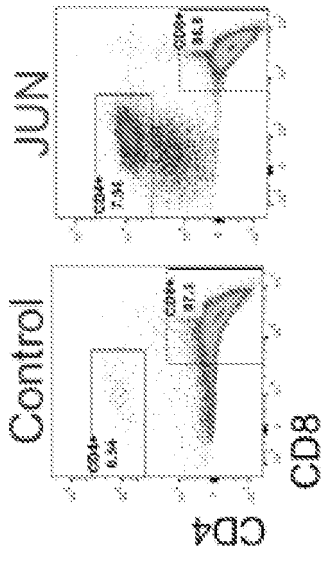


FIG. 14J

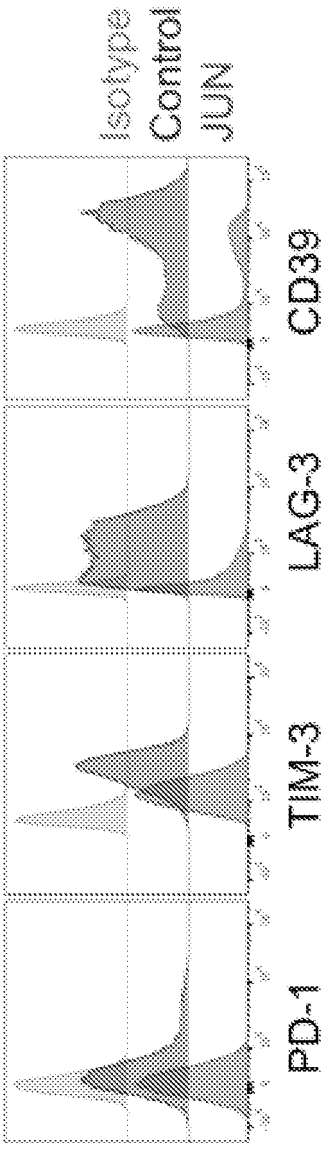


FIG. 15A

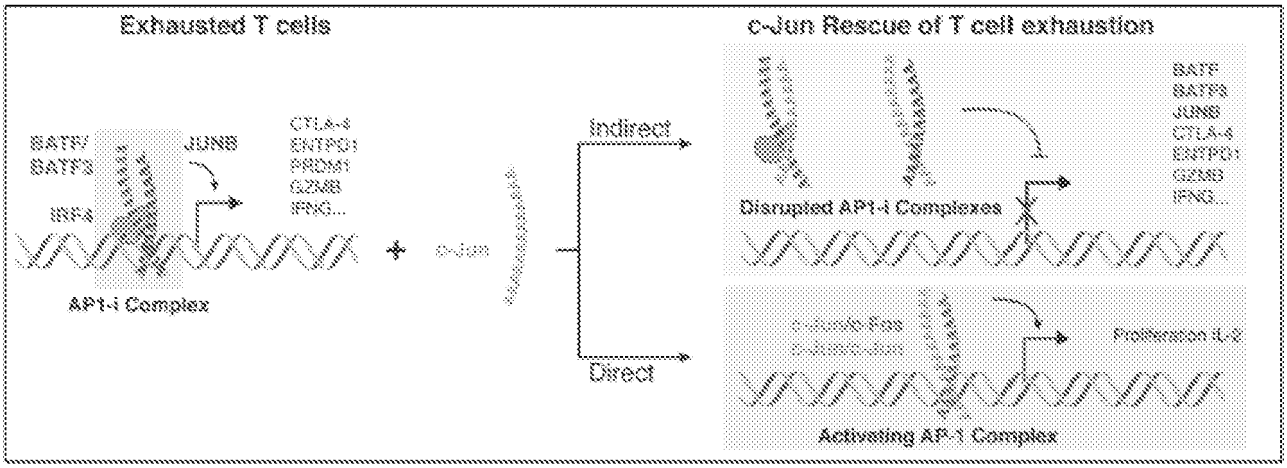


FIG. 15B



FIG. 15C

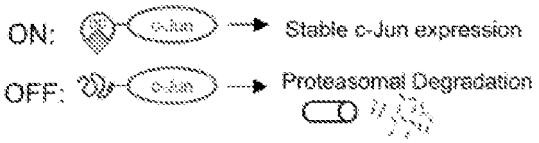


FIG. 15D

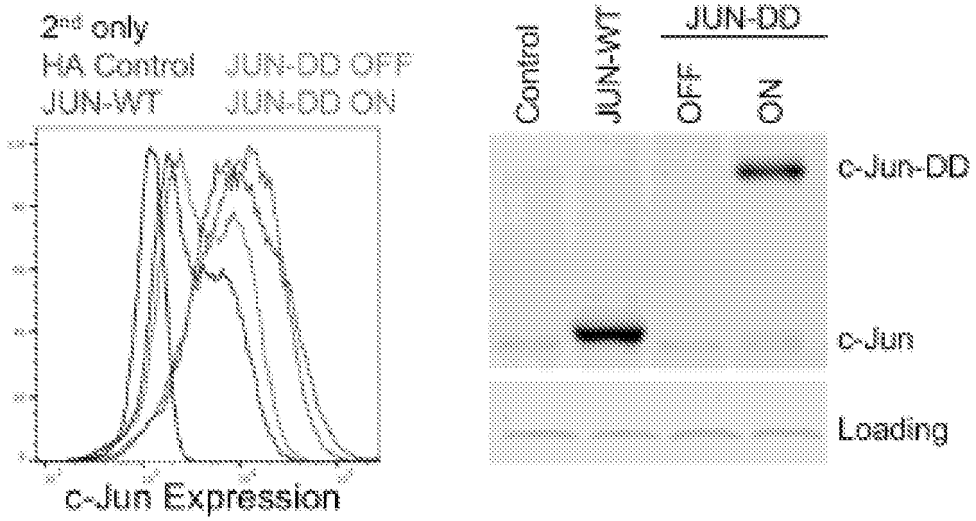


FIG. 15E

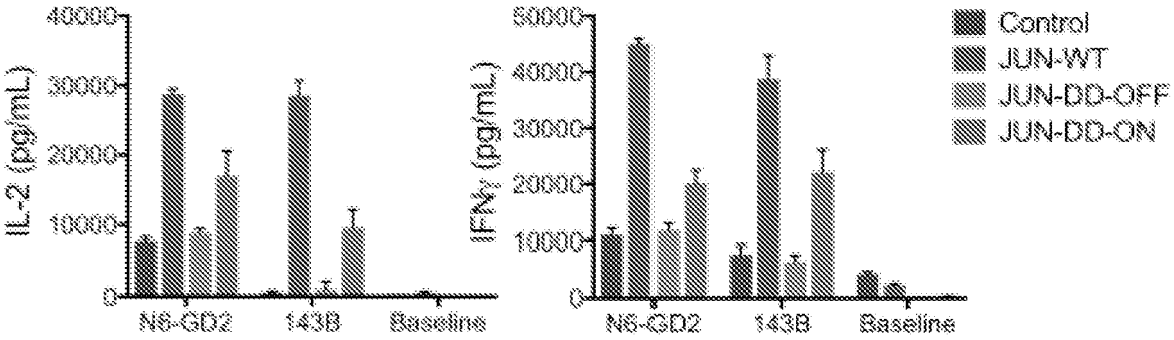


FIG. 15F

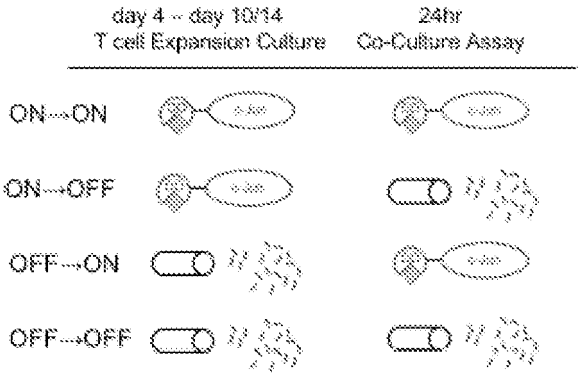


FIG. 15G

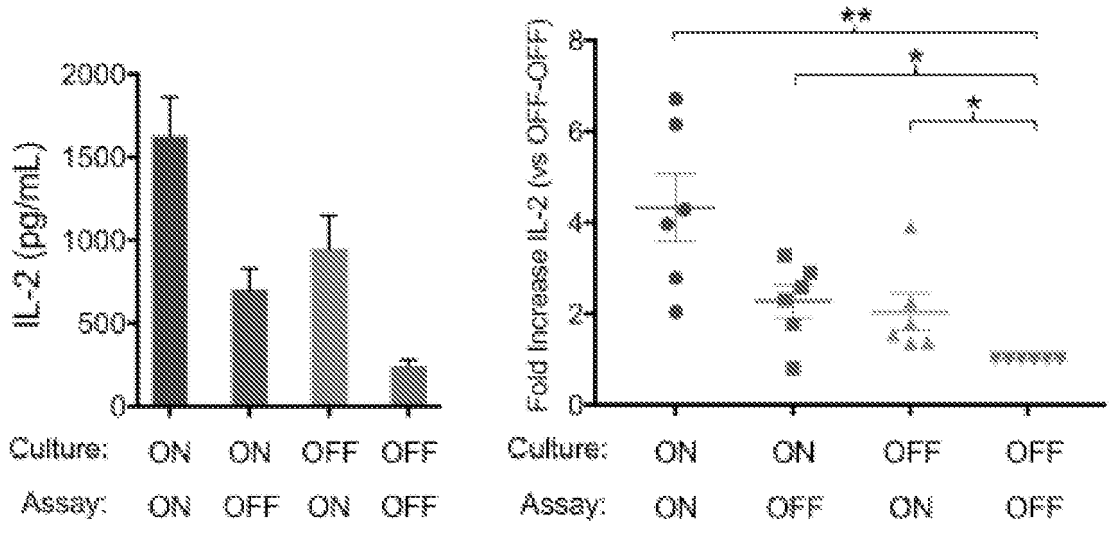


FIG. 15H

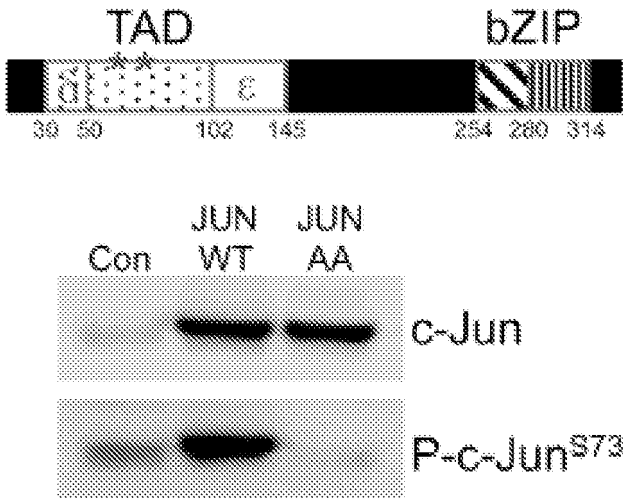


FIG. 15I

FIG. 15J

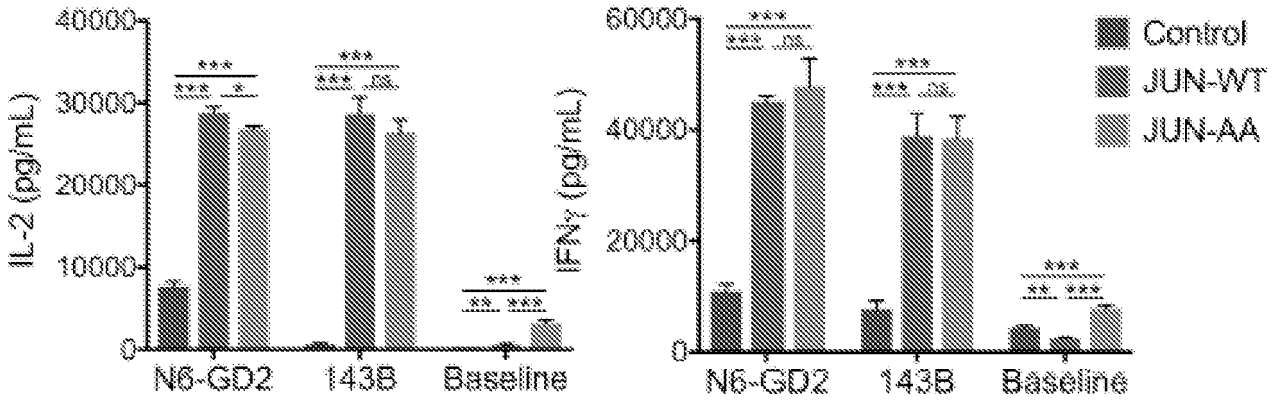


FIG. 16A

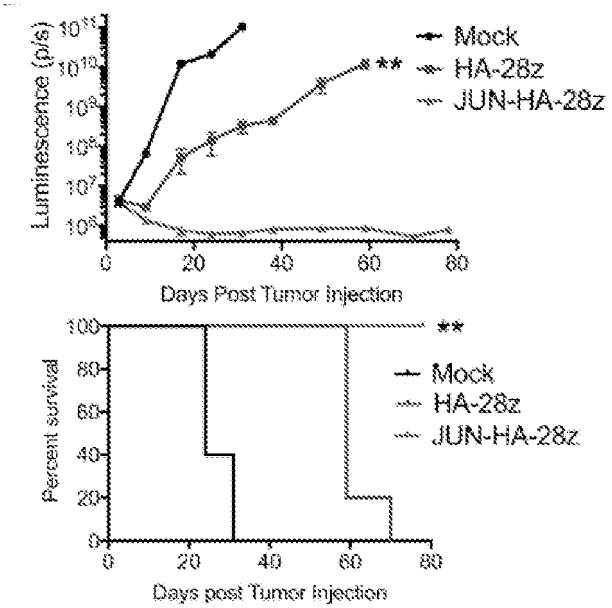


FIG. 16B

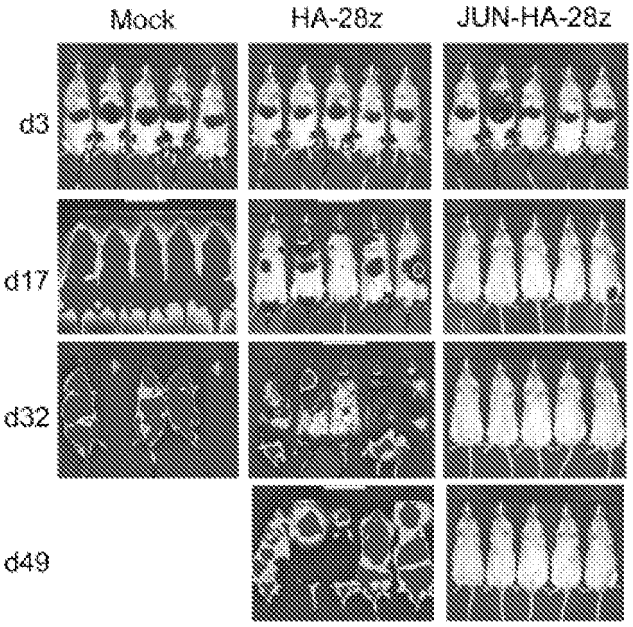


FIG. 16C



FIG. 16D

FIG. 16E

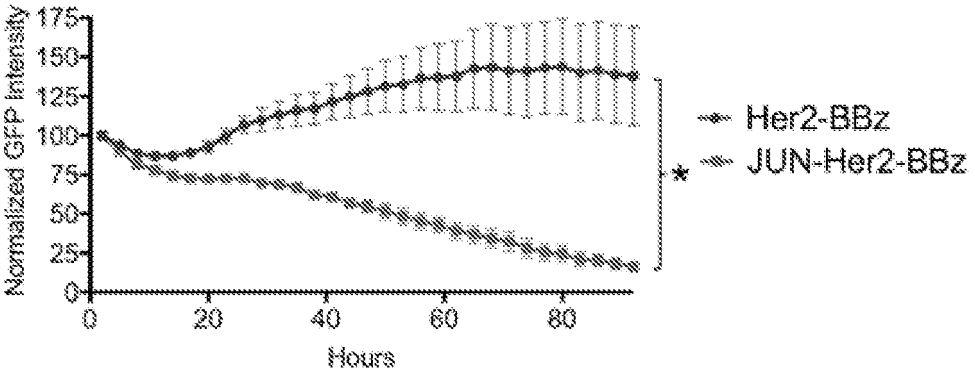
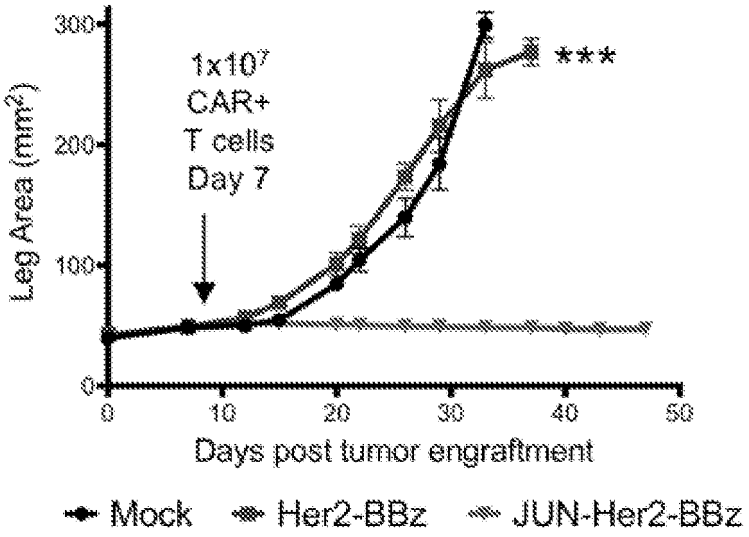


FIG. 16F



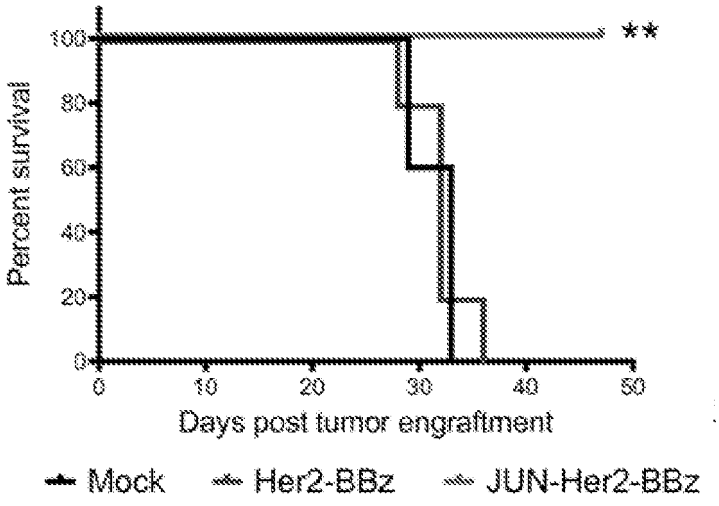


FIG. 16G

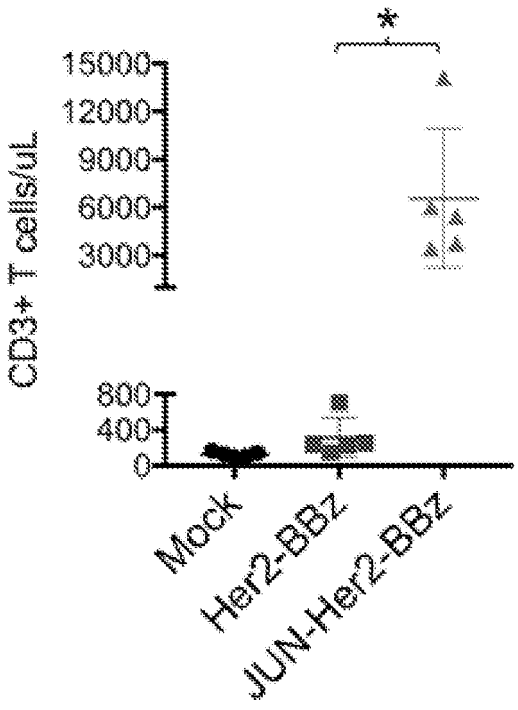


FIG. 16H

FIG. 17A

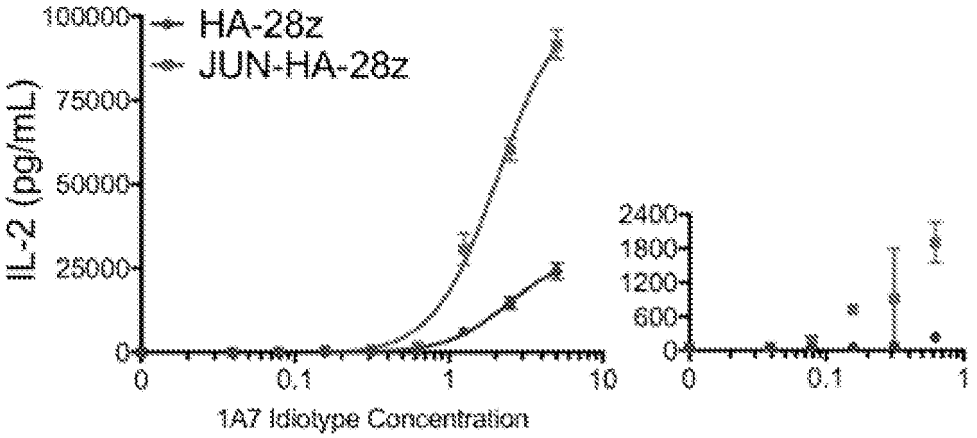


FIG. 17B

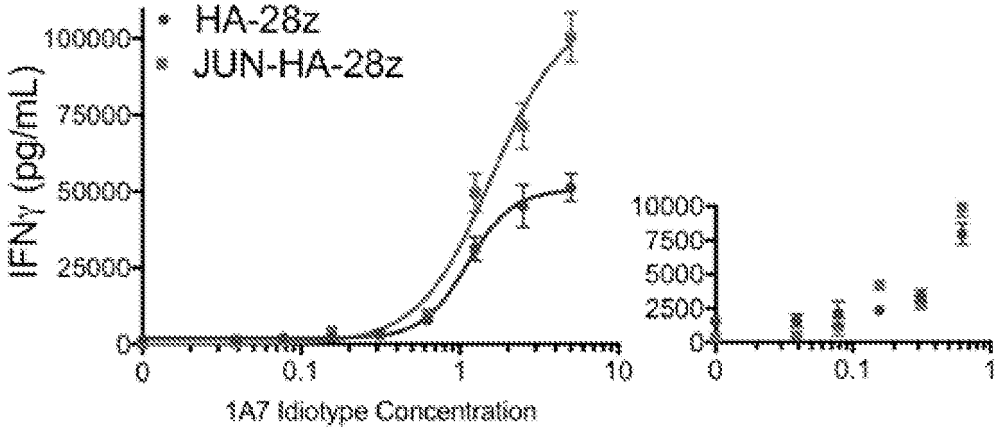




FIG. 17C

FIG. 17D

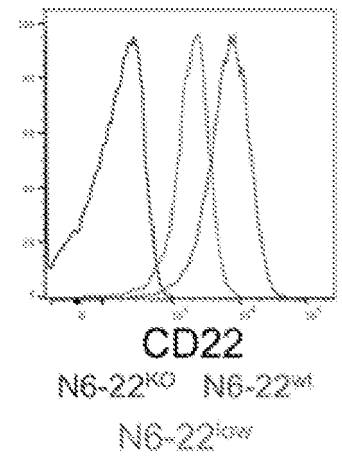


FIG. 17E

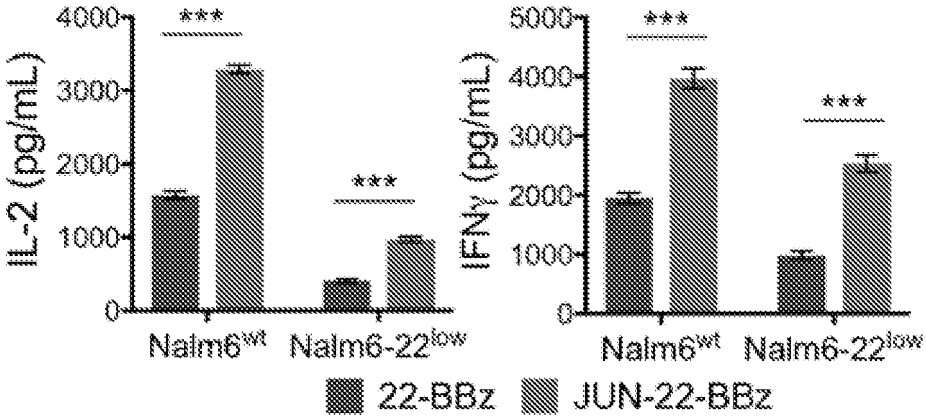


FIG. 17F

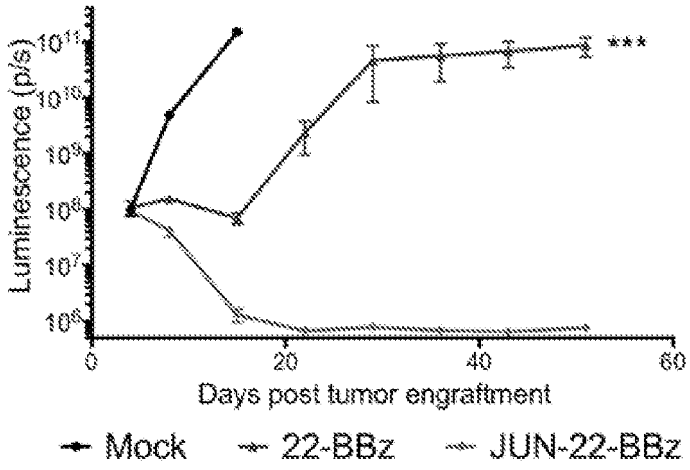


FIG. 17G

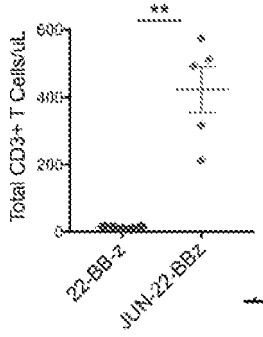


FIG. 17H

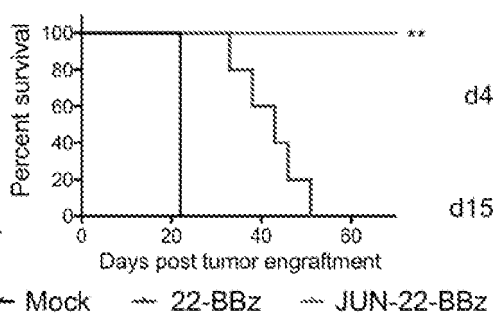


FIG. 17I

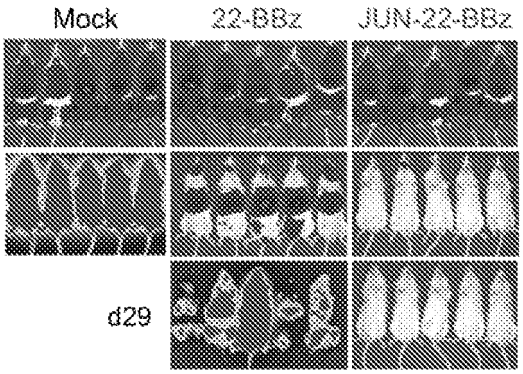


FIG. 18A

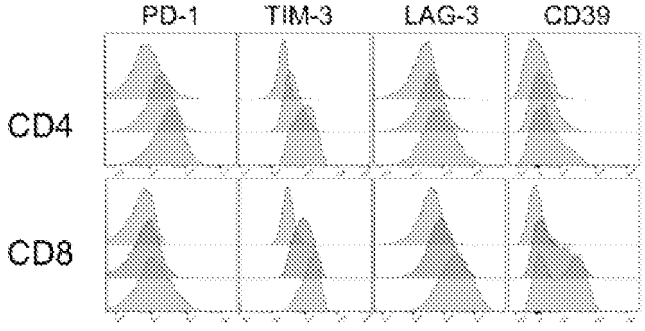
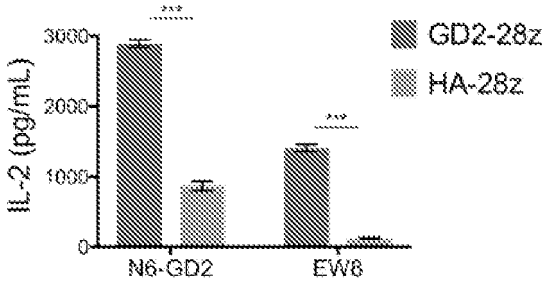
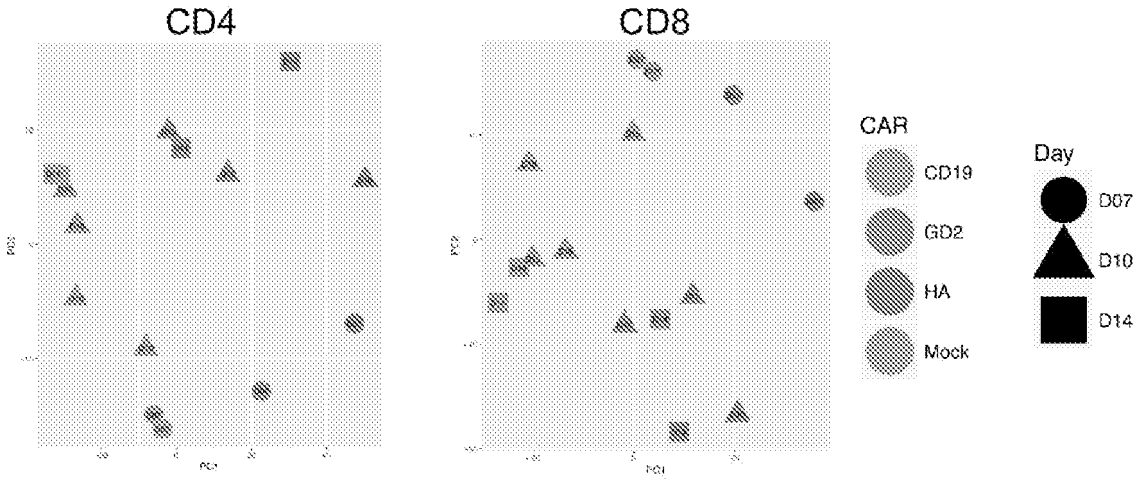


FIG. 18B



CD19-28z GD2-28Z HA-28z

FIG. 18C



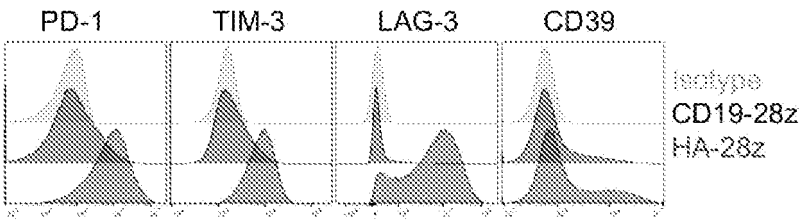


FIG. 18D

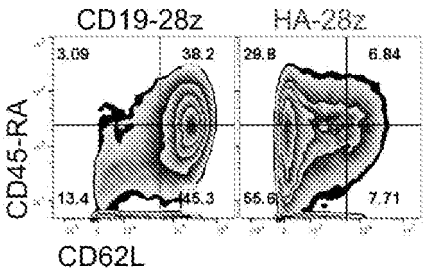


FIG. 18E

FIG. 18F

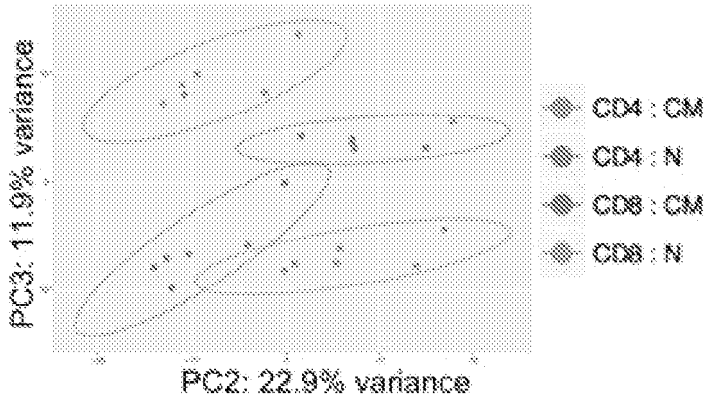
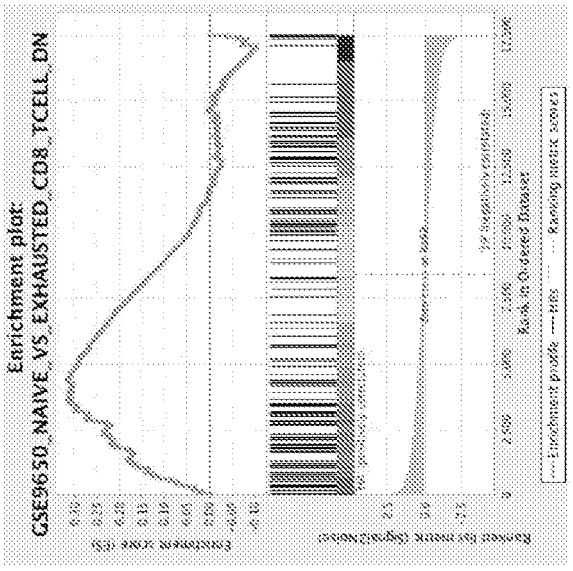
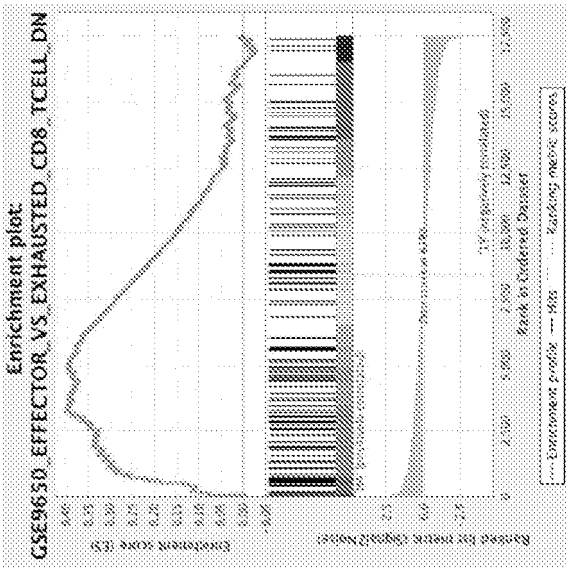


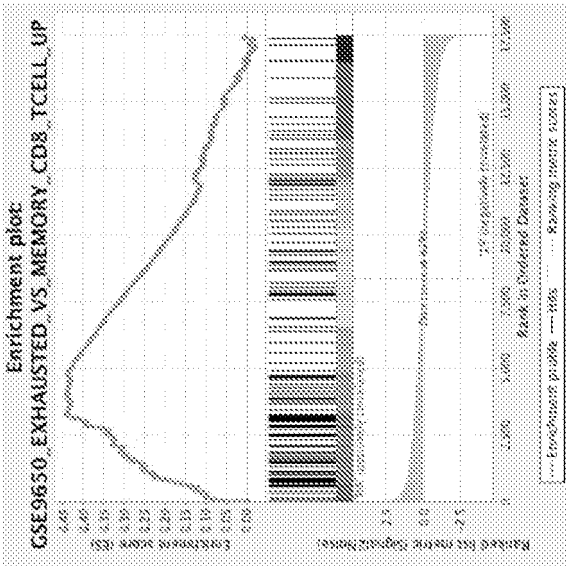
FIG. 18G



NES	1.530
Nom p-value	0.00265
FDR q-value	0.00816



NES	1.862
Nom p-value	0.0
FDR q-value	2.06E-4



NES	2.0977
Nom p-value	0.0
FDR q-value	4.246E-6

FIG. 19B

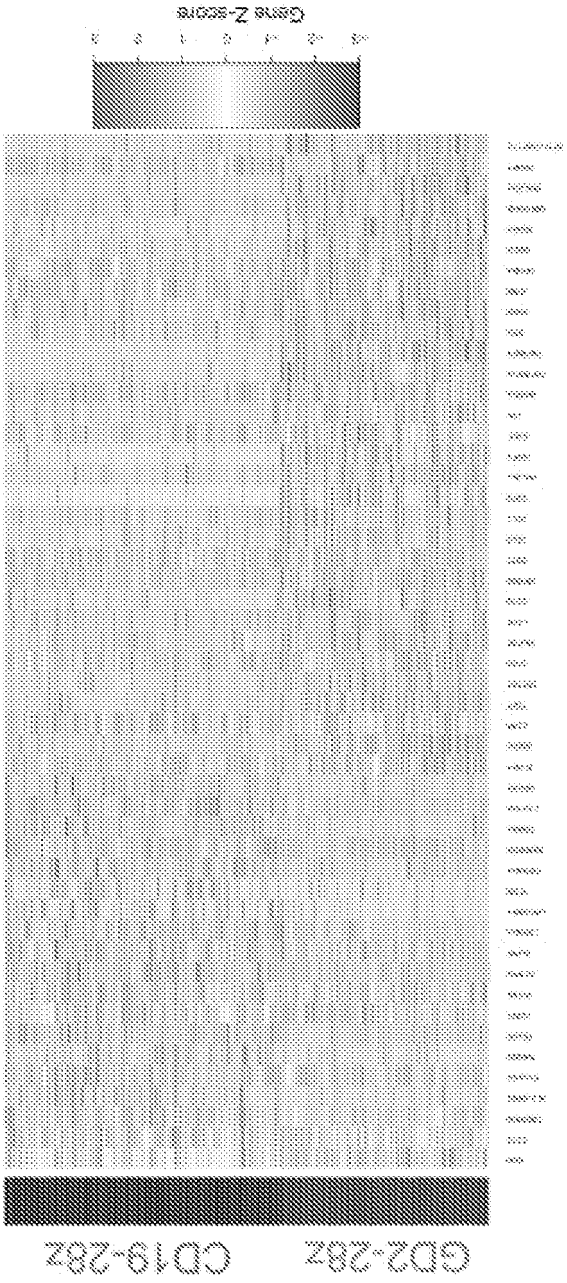


FIG. 19A

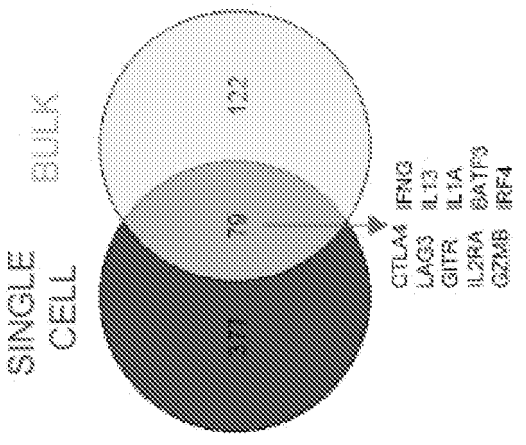


FIG. 19C

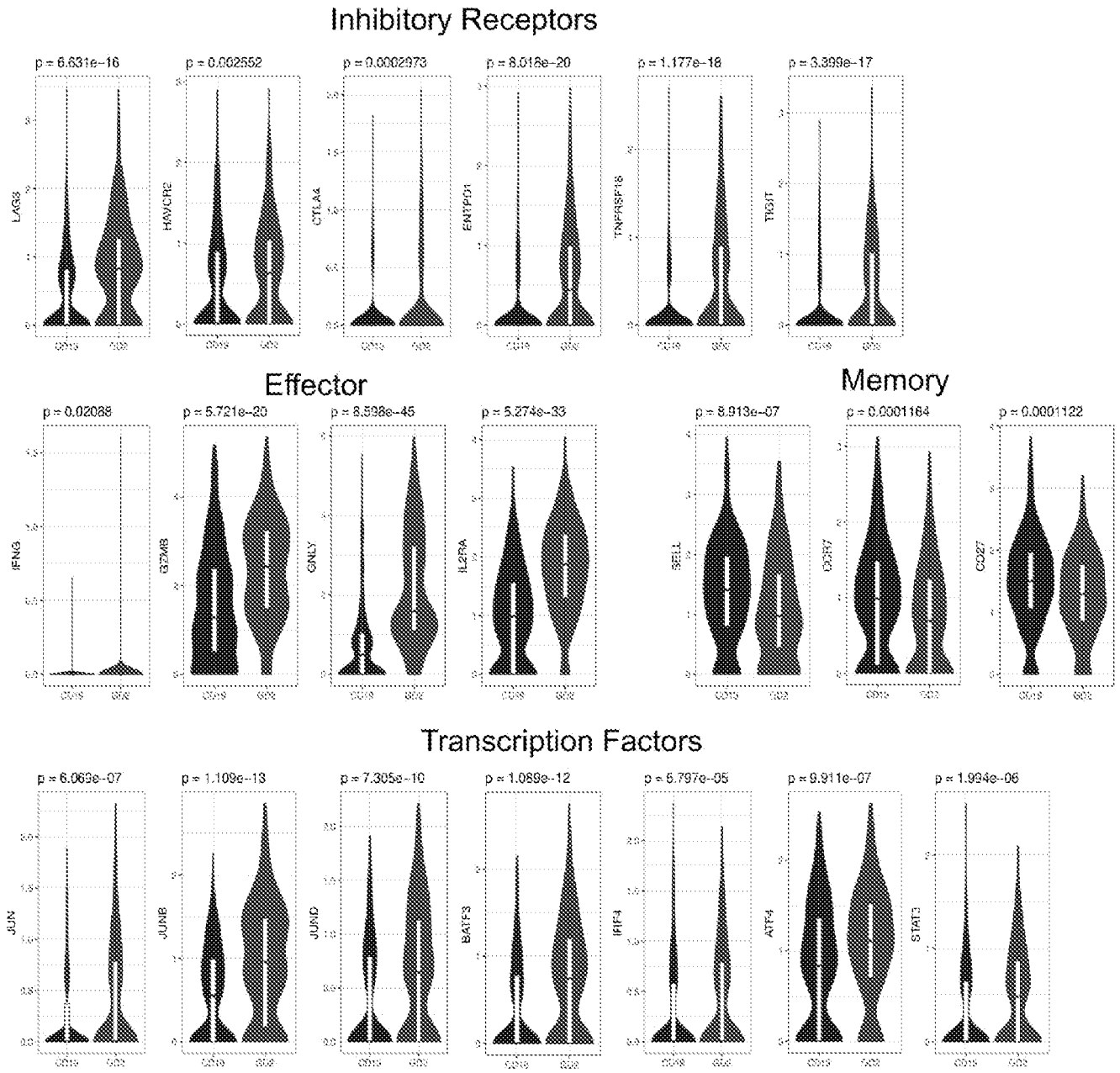

Inhibitory Receptors
p = 6.631e−16
LAG3
p = 0.002552
HAVCR2
p = 0.0002973
CTLA4
p = 8.018e−20
ENTPD1
p = 1.177e−18
TNFRSF18
p = 3.399e−17
TIGIT
Effector
p = 0.02088
IFNG
p = 5.721e−20
GZMB
p = 8.598e−45
GNLY
p = 5.274e−33
IL2RA
Memory
p = 8.913e−07
SELL
p = 0.0001164
CCR7
p = 0.0001122
CD27
Transcription Factors
p = 6.069e−07
JUN
p = 1.109e−13
JUNB
p = 7.305e−10
JUND
p = 1.089e−12
BATF3
p = 5.797e−05
IRF4
p = 9.911e−07
ATF4
p = 1.994e−06
STAT3

FIG. 20A

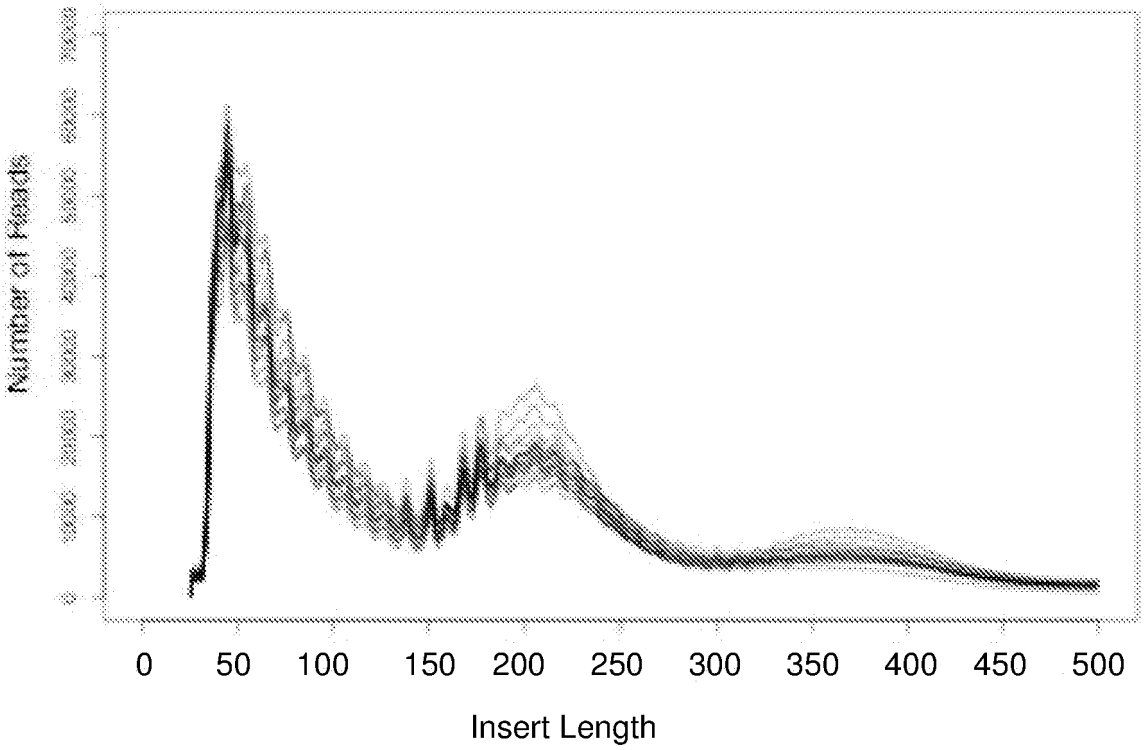


FIG. 20B

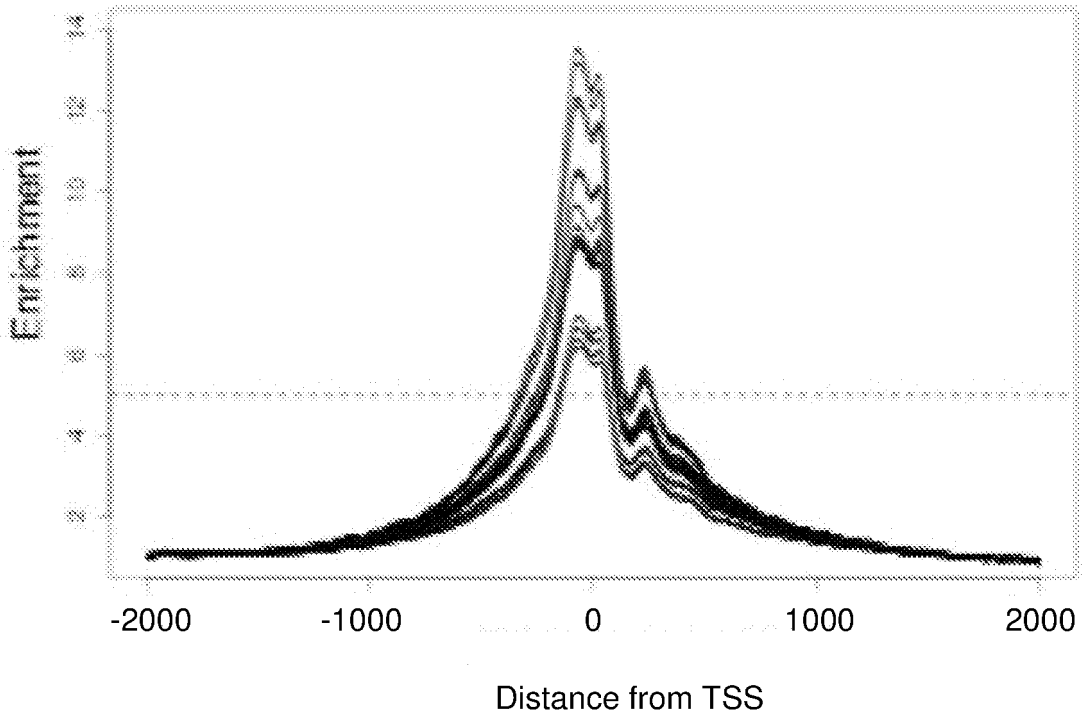


FIG. 20C

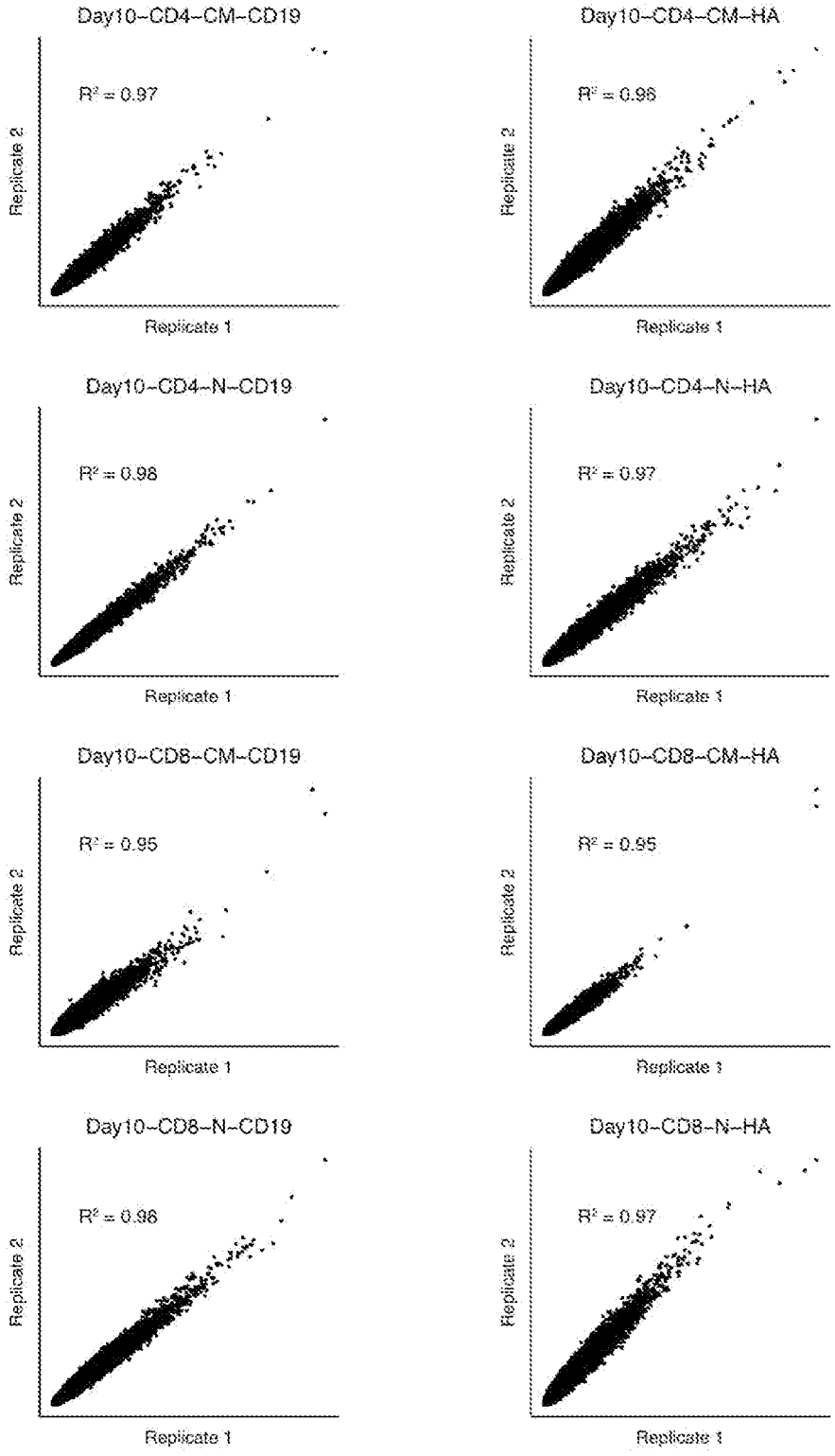

Day10-CD4-CM-CD19
R² = 0.97
Replicate 2
Replicate 1
Day10-CD4-CM-HA
R² = 0.96
Replicate 2
Replicate 1
Day10-CD4-N-CD19
R² = 0.98
Replicate 2
Replicate 1
Day10-CD4-N-HA
R² = 0.97
Replicate 2
Replicate 1
Day10-CD8-CM-CD19
R² = 0.95
Replicate 2
Replicate 1
Day10-CD8-CM-HA
R² = 0.95
Replicate 2
Replicate 1
Day10-CD8-N-CD19
R² = 0.96
Replicate 2
Replicate 1
Day10-CD8-N-HA
R² = 0.97
Replicate 2
Replicate 1

FIG. 20D

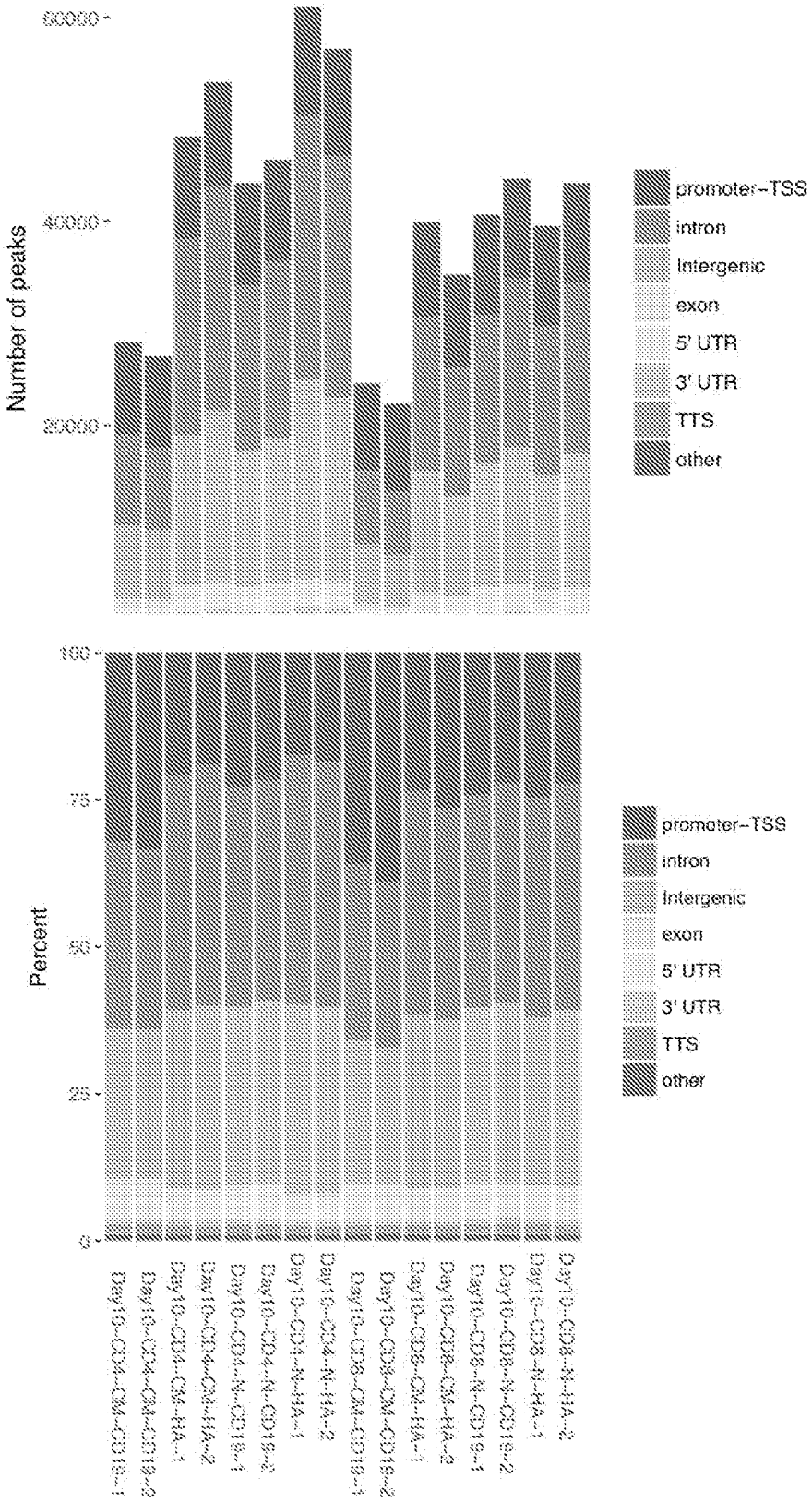

60000
40000
20000
Number of peaks
promoter-TSS
intron
Intergenic
exon
5' UTR
3' UTR
TTS
other
100
75
50
25
0
Percent
promoter-TSS
intron
Intergenic
exon
5' UTR
3' UTR
TTS
other
Day10-CD4-CM-CD19-1
Day10-CD4-CM-CD19-2
Day10-CD4-CM-HA-1
Day10-CD4-CM-HA-2
Day10-CD4-N-CD19-1
Day10-CD4-N-CD19-2
Day10-CD4-N-HA-1
Day10-CD4-N-HA-2
Day10-CD8-CM-CD19-1
Day10-CD8-CM-CD19-2
Day10-CD8-CM-HA-1
Day10-CD8-CM-HA-2
Day10-CD8-N-CD19-1
Day10-CD8-N-CD19-2
Day10-CD8-N-HA-1
Day10-CD8-N-HA-2

FIG. 21A

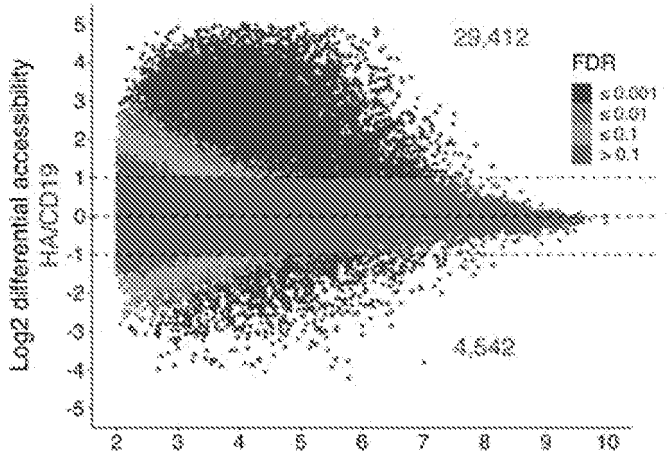


FIG. 21B

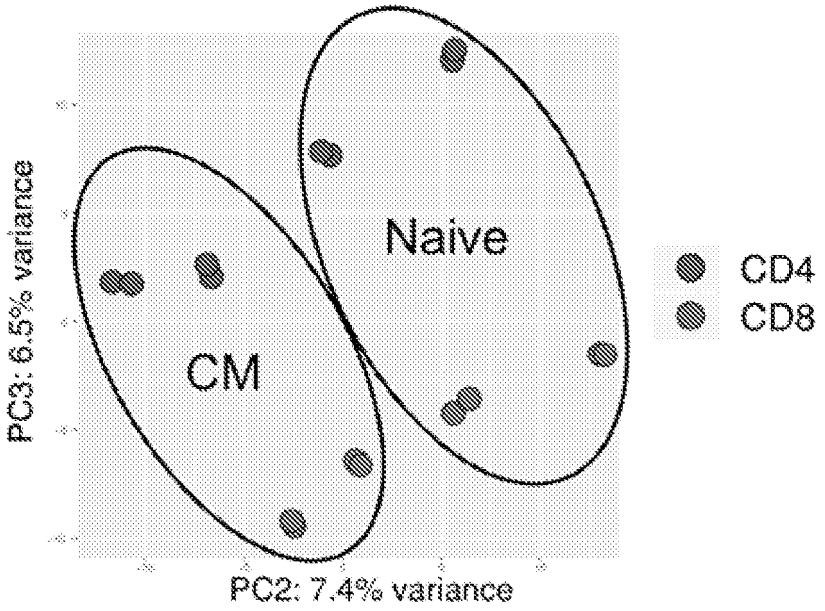
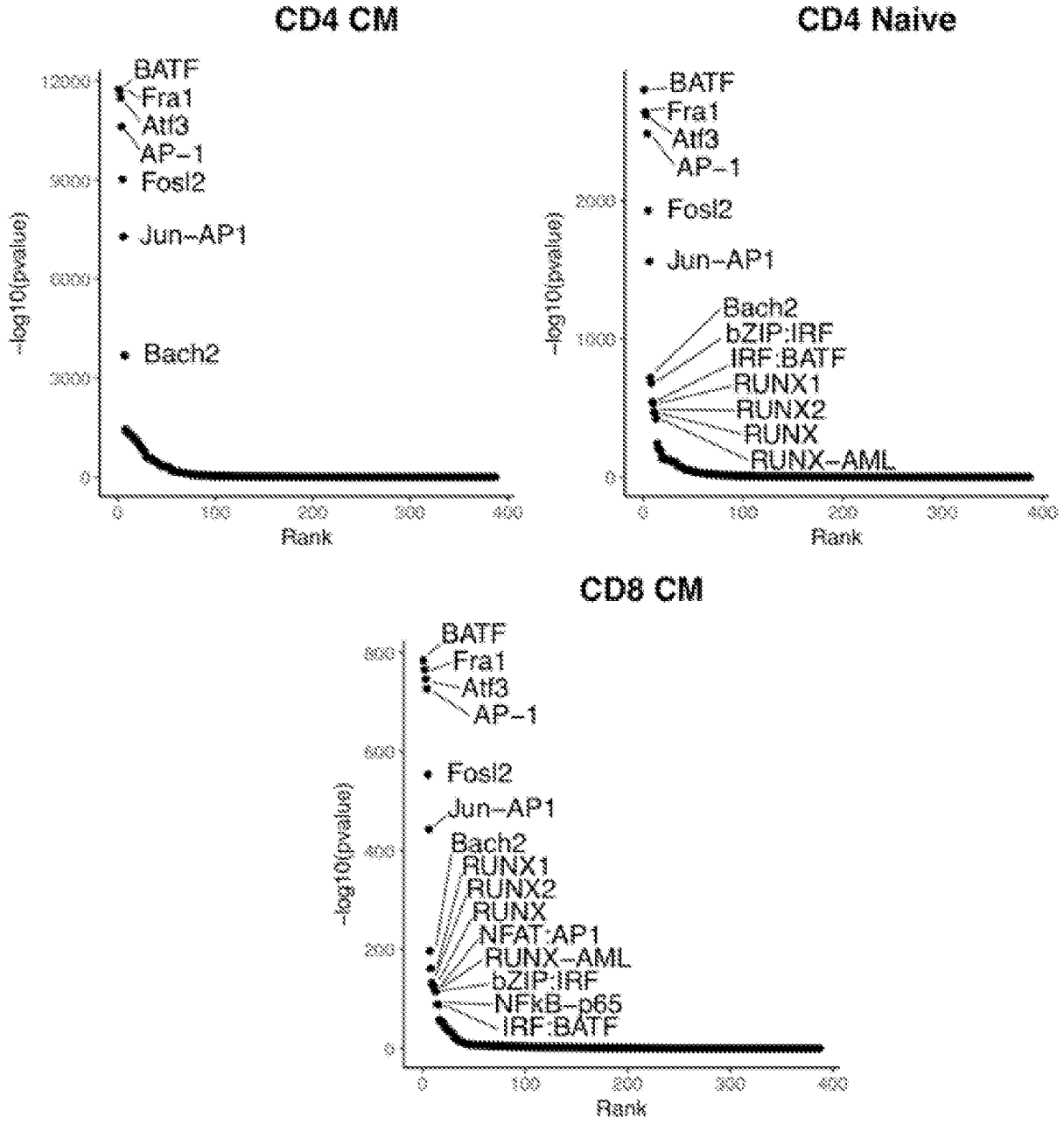


FIG. 21C


CD4 CM
BATF
Fra1
Atf3
AP-1
Fosl2
Jun-AP1
Bach2
-log10(pvalue)
Rank
CD4 Naive
BATF
Fra1
Atf3
AP-1
Fosl2
Jun-AP1
Bach2
bZIP:IRF
IRF:BATF
RUNX1
RUNX2
RUNX
RUNX-AML
-log10(pvalue)
Rank
CD8 CM
BATF
Fra1
Atf3
AP-1
Fosl2
Jun-AP1
Bach2
RUNX1
RUNX2
RUNX
NFAT:AP1
RUNX-AML
bZIP:IRF
NFkB-p65
IRF:BATF
-log10(pvalue)
Rank

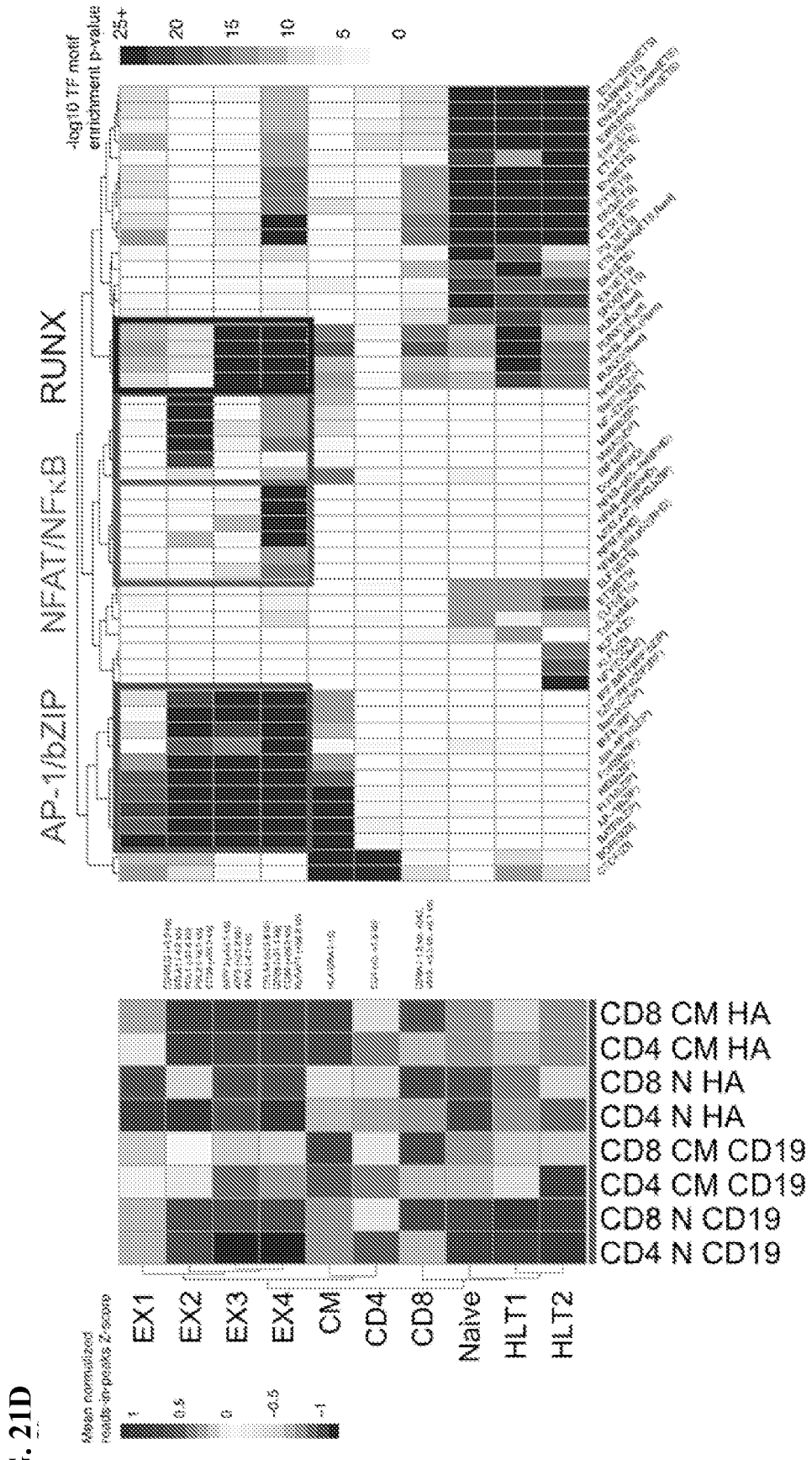

AP-1/bZIP
NFAT/NFκB
RUNX
-log10 TF motif enrichment p-value
25+
20
15
10
5
0
CD8 CM HA
CD4 CM HA
CD8 N HA
CD4 N HA
CD8 CM CD19
CD4 CM CD19
CD8 N CD19
CD4 N CD19
EX1
EX2
EX3
EX4
CM
CD4
CD8
Naive
HLT1
HLT2
Mean normalized peaks in peaks Z-score
1
0.5
0
-0.5
-1

FIG. 21D

FIG. 22A

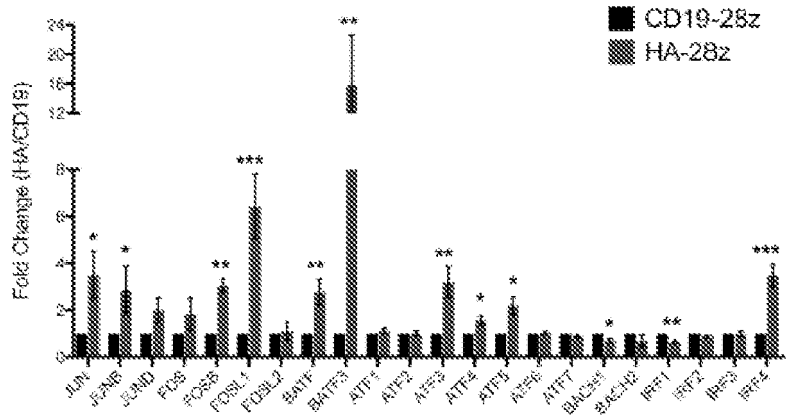
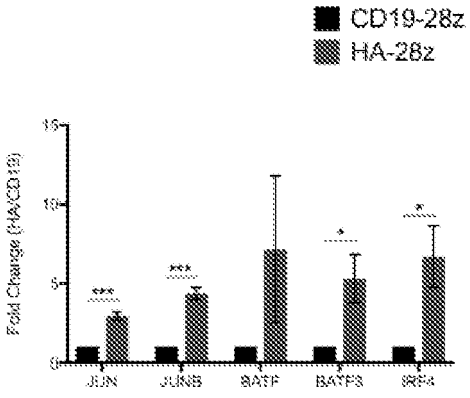


FIG. 22B



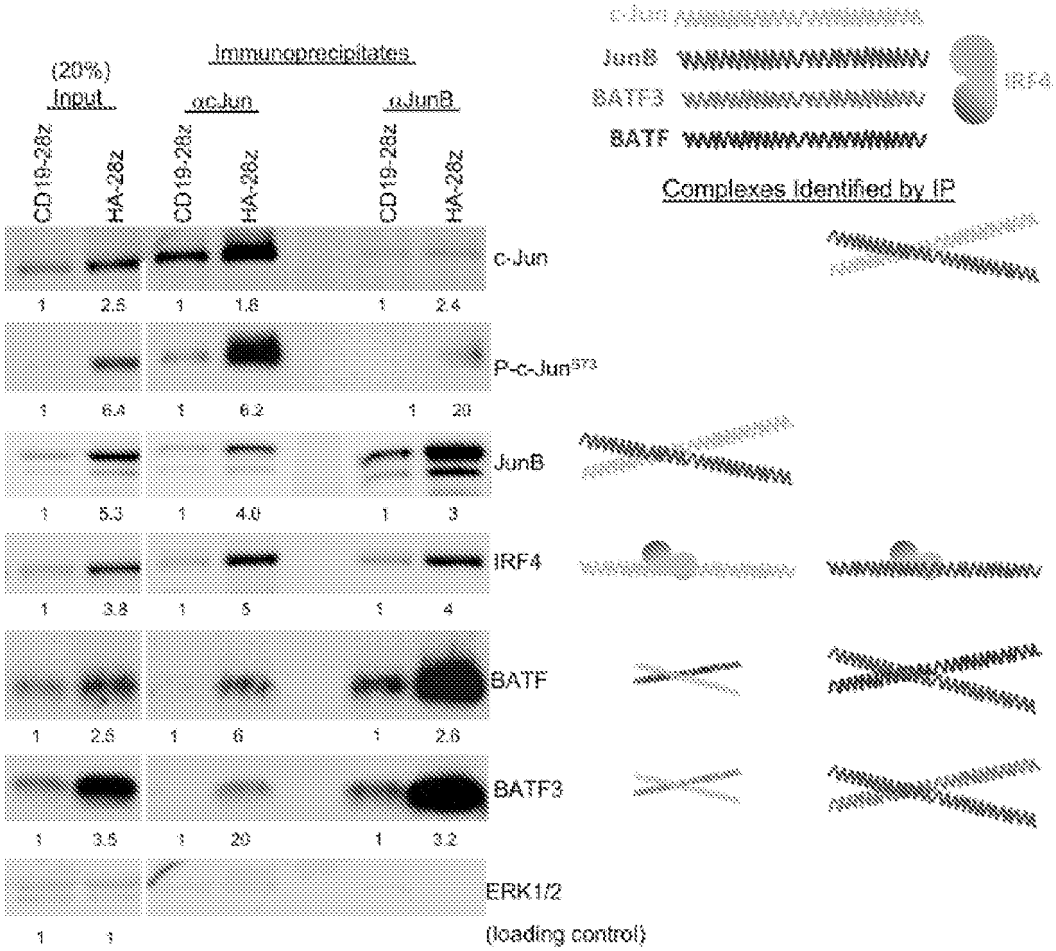


FIG. 22C

FIG. 23A



FIG. 23B

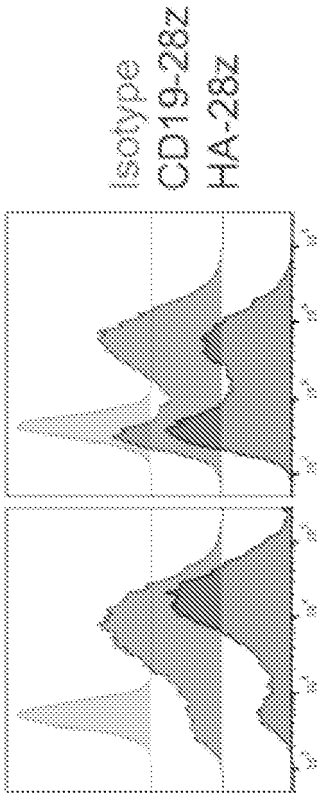


FIG. 23C

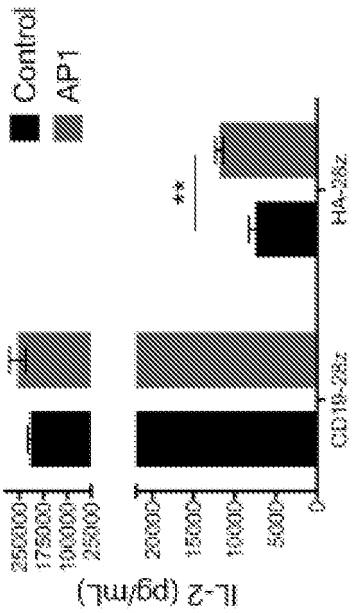


FIG. 23D



FIG. 23E

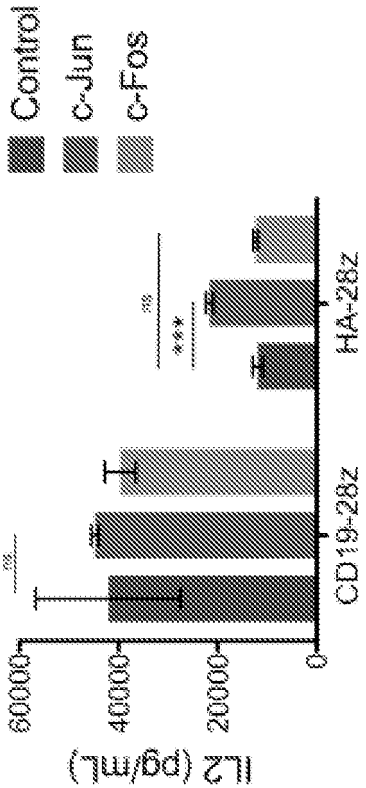


FIG. 24A

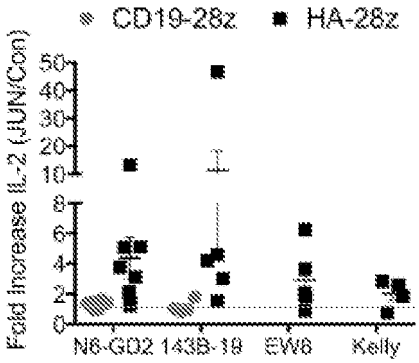


FIG. 24B

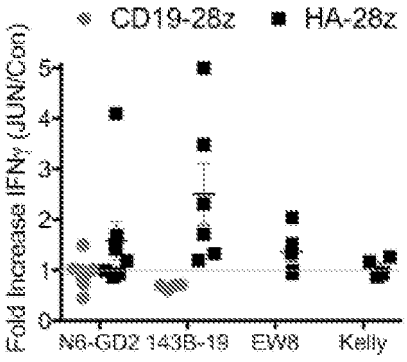


FIG. 24C

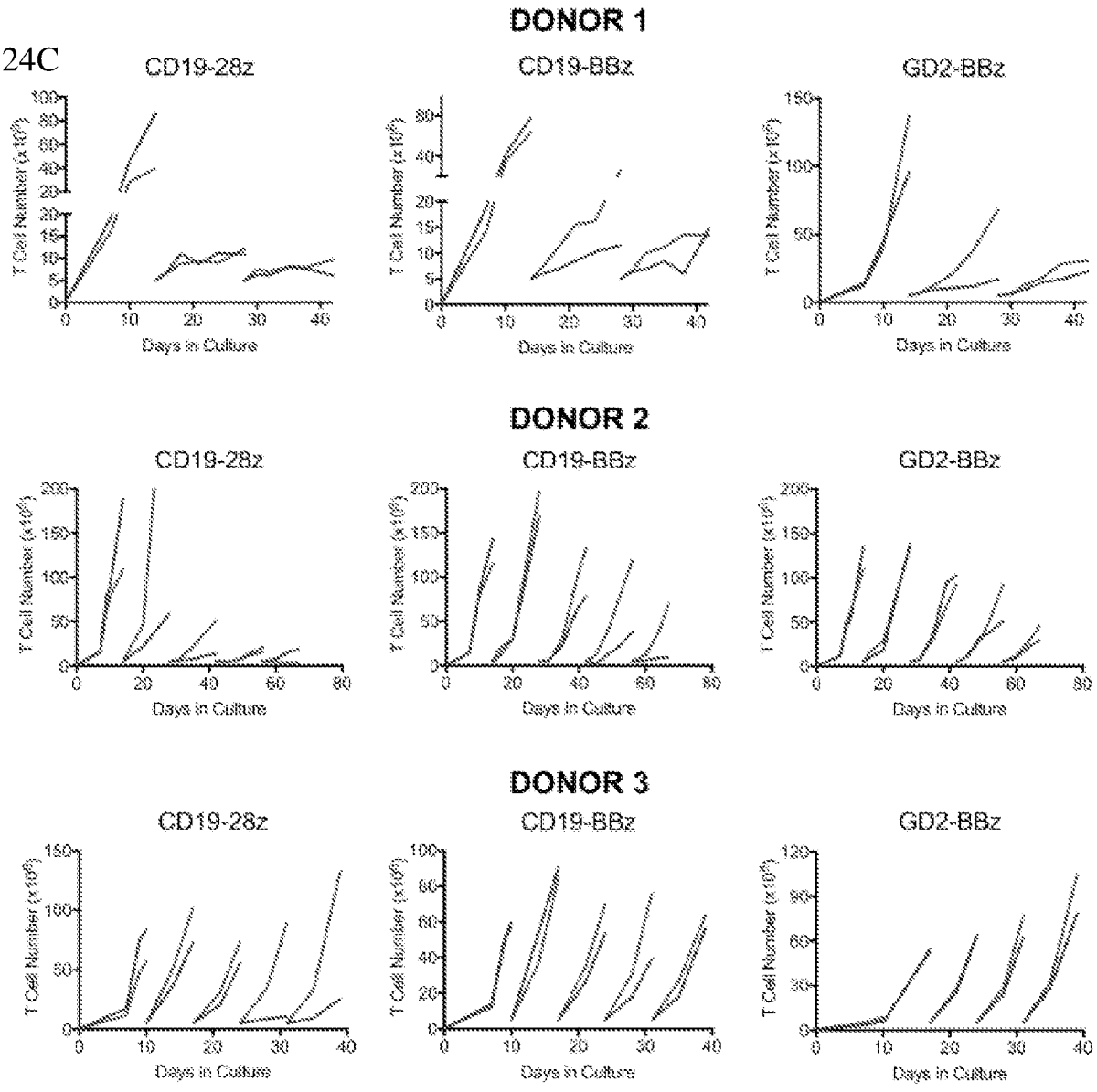


FIG. 25A

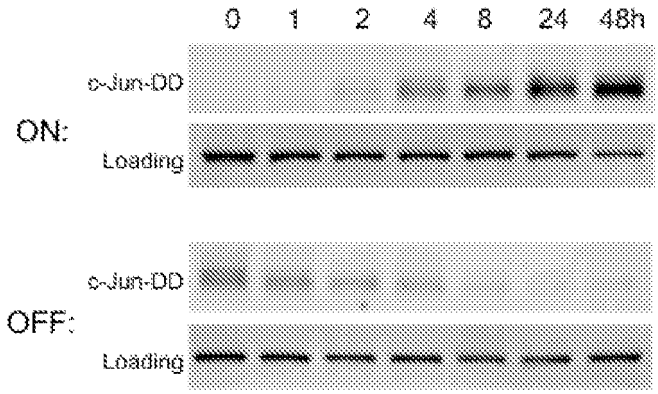


FIG. 25B

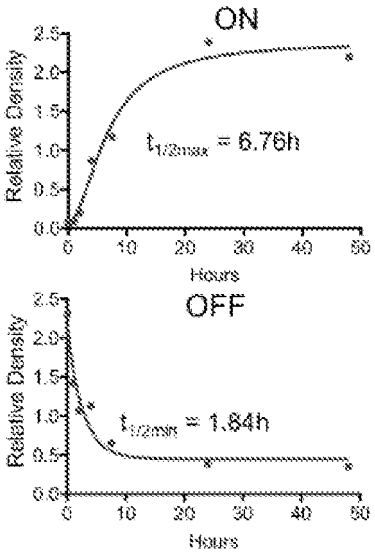


FIG. 25C

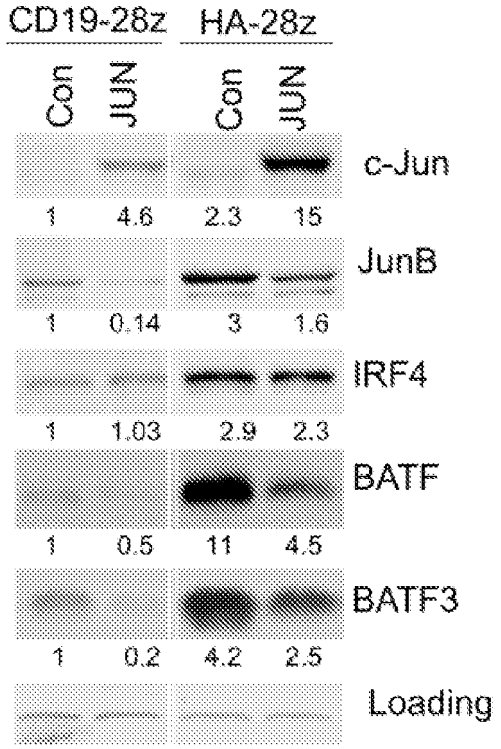
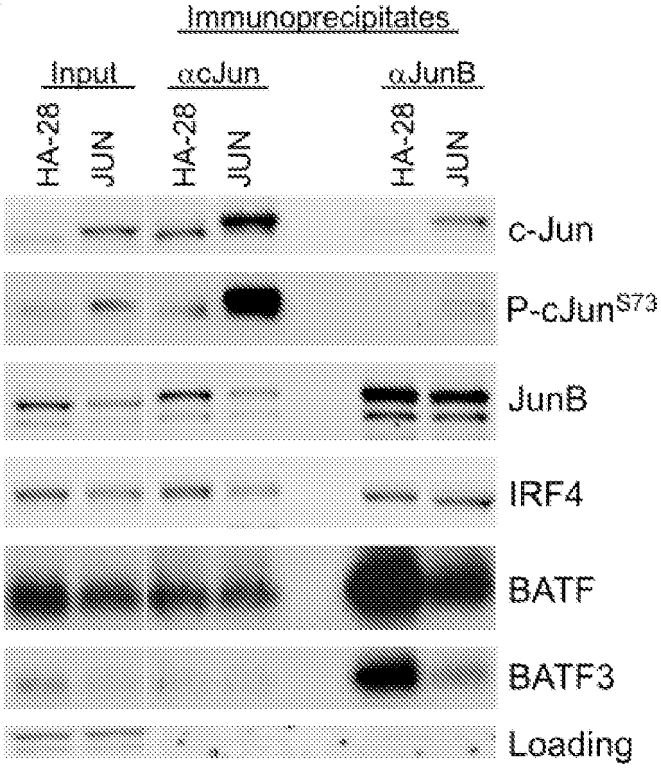


FIG. 25E



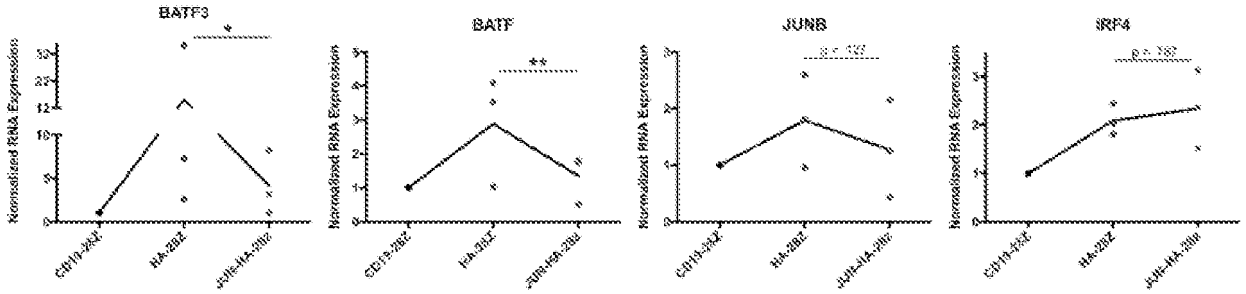


FIG. 25D

FIG. 26A



FIG. 26B

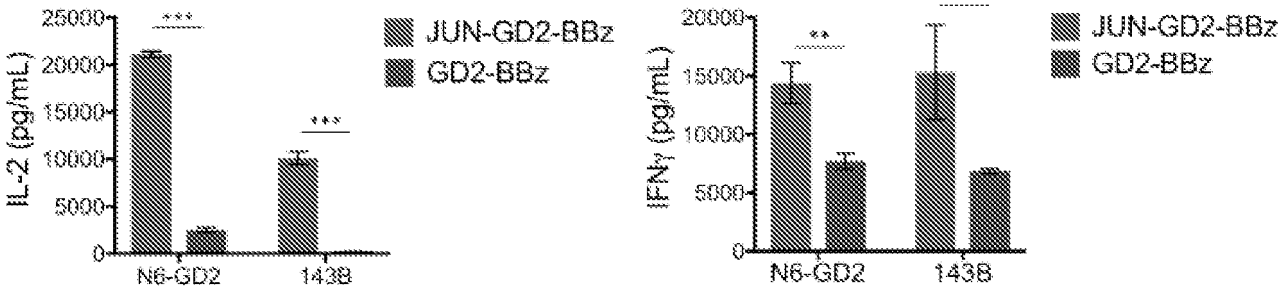


FIG. 26C

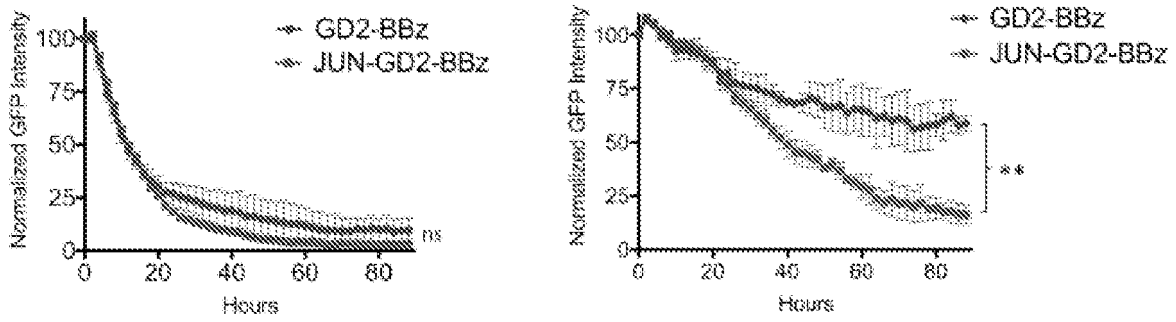


FIG. 26D

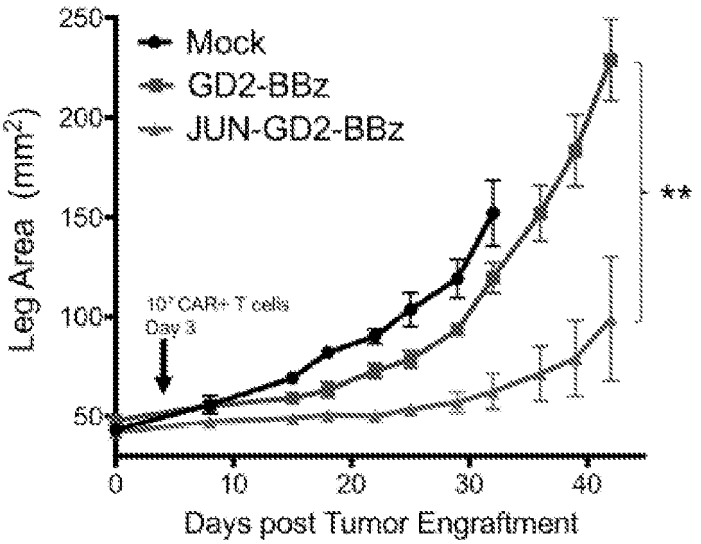


FIG. 26E

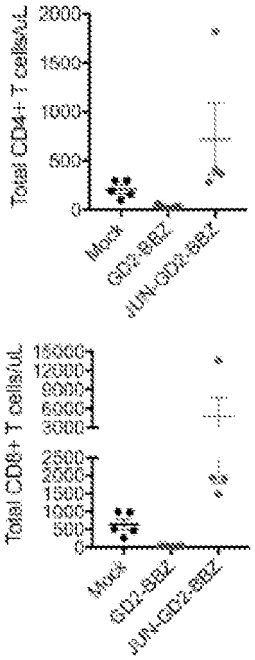


FIG. 27A

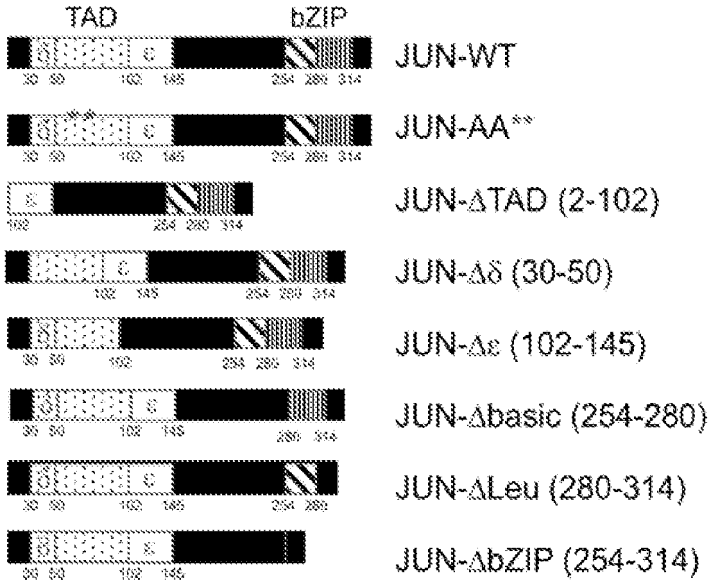


FIG. 27B

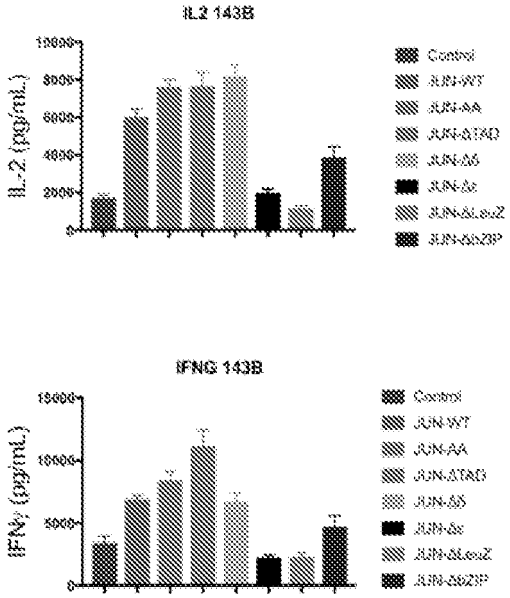


FIG. 27C

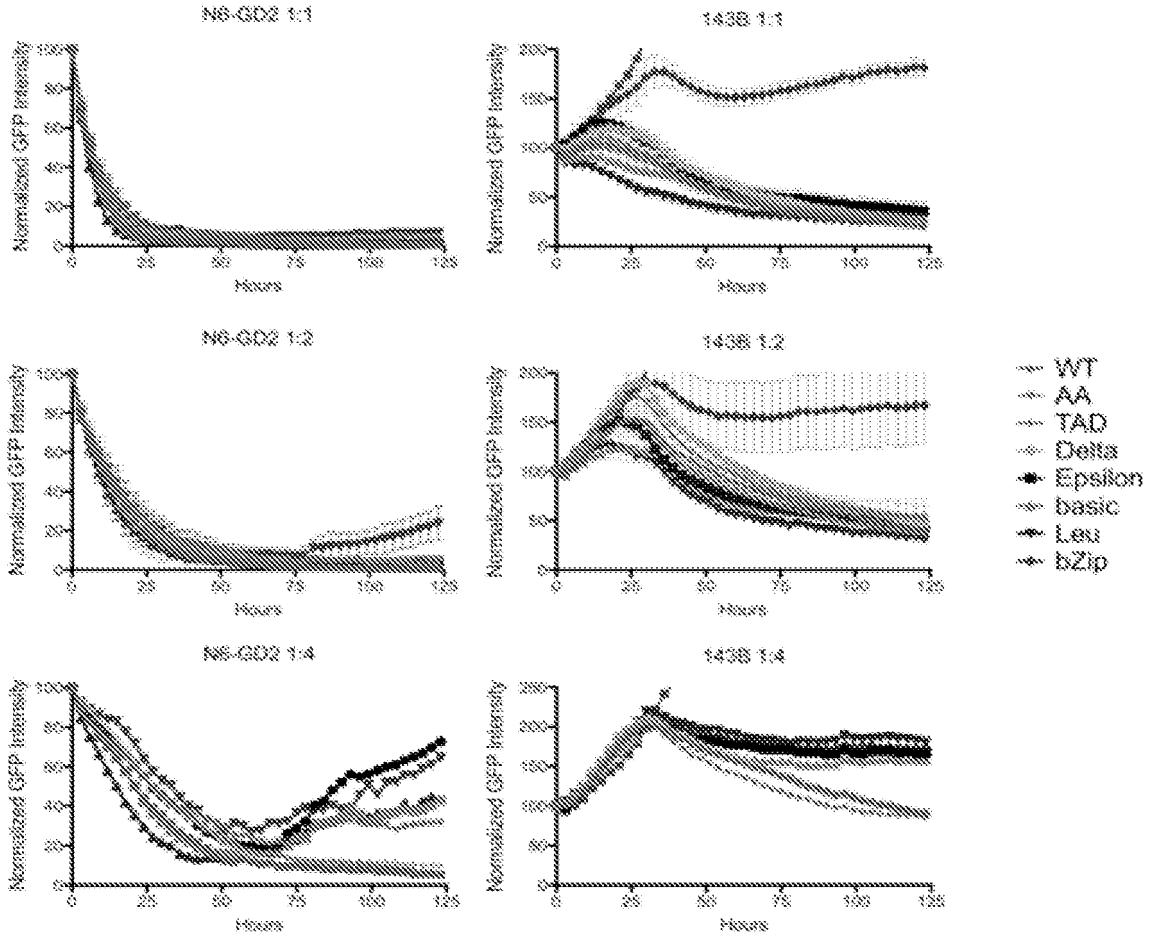


FIG. 27D

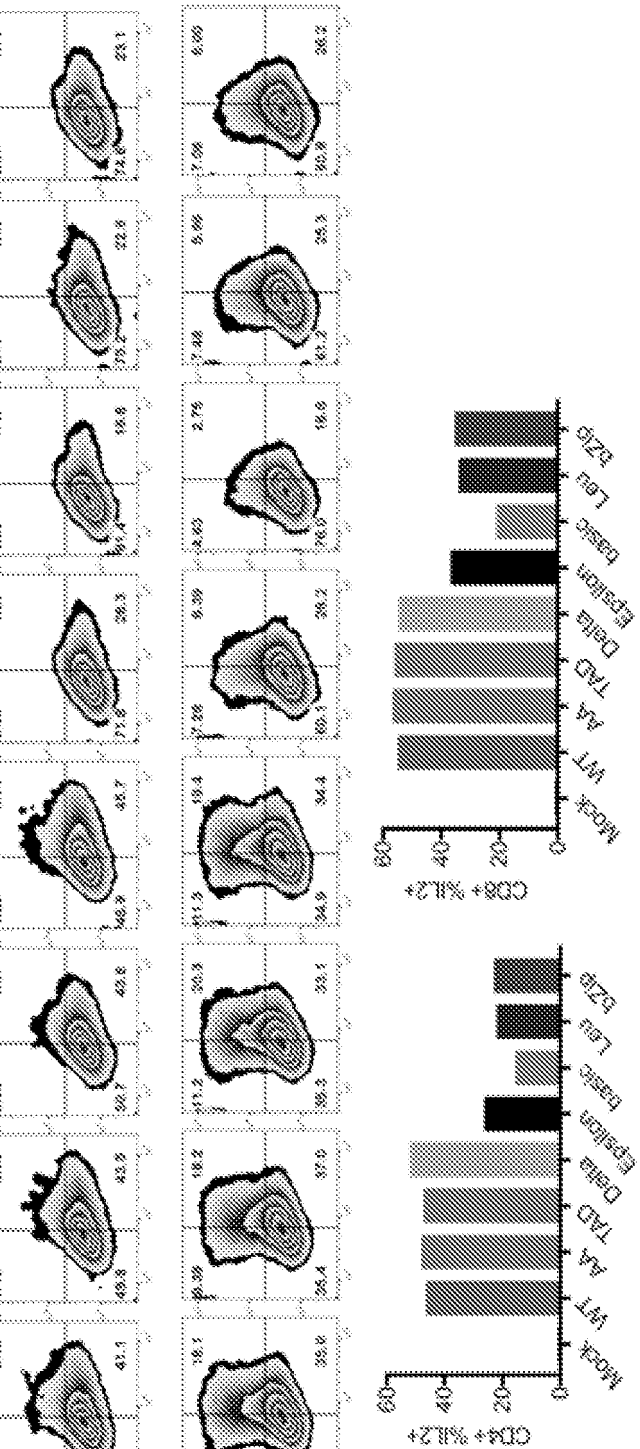


FIG. 27E

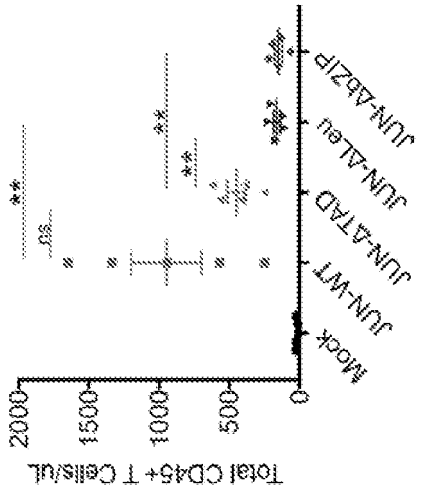


FIG. 28

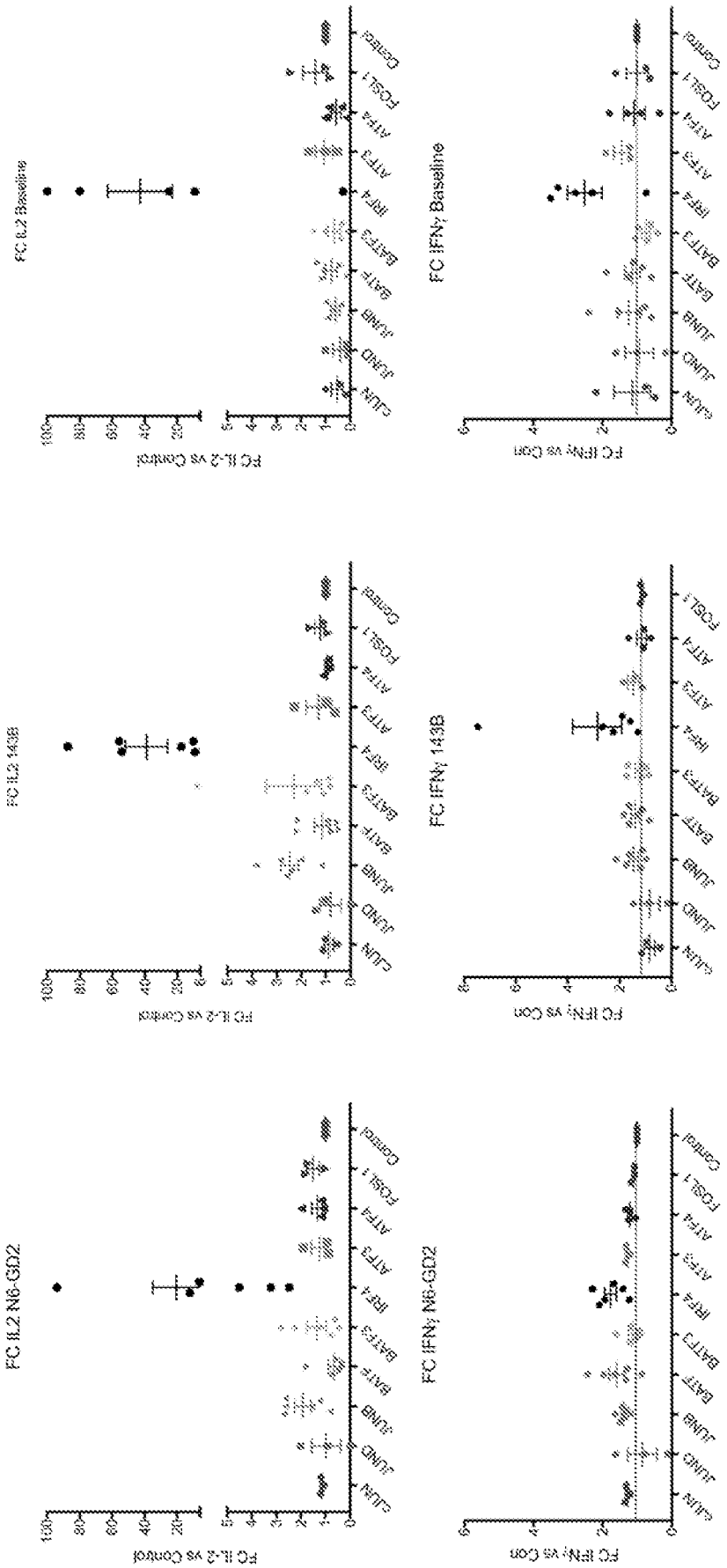

FC IL2 Baseline
FC IL-2 vs Control
FC IFNγ Baseline
FC IFNγ vs Con
FC IL2 143B
FC IL-2 vs Control
FC IFNγ 143B
FC IFNγ vs Con
FC IL2 N6-GD2
FC IL-2 vs Control
FC IFNγ N6-GD2
FC IFNγ vs Con
CJUN
JUND
JUNB
BATF
BATF3
IRF4
ATF3
ATF4
FOSL1
Control

FIG. 29A

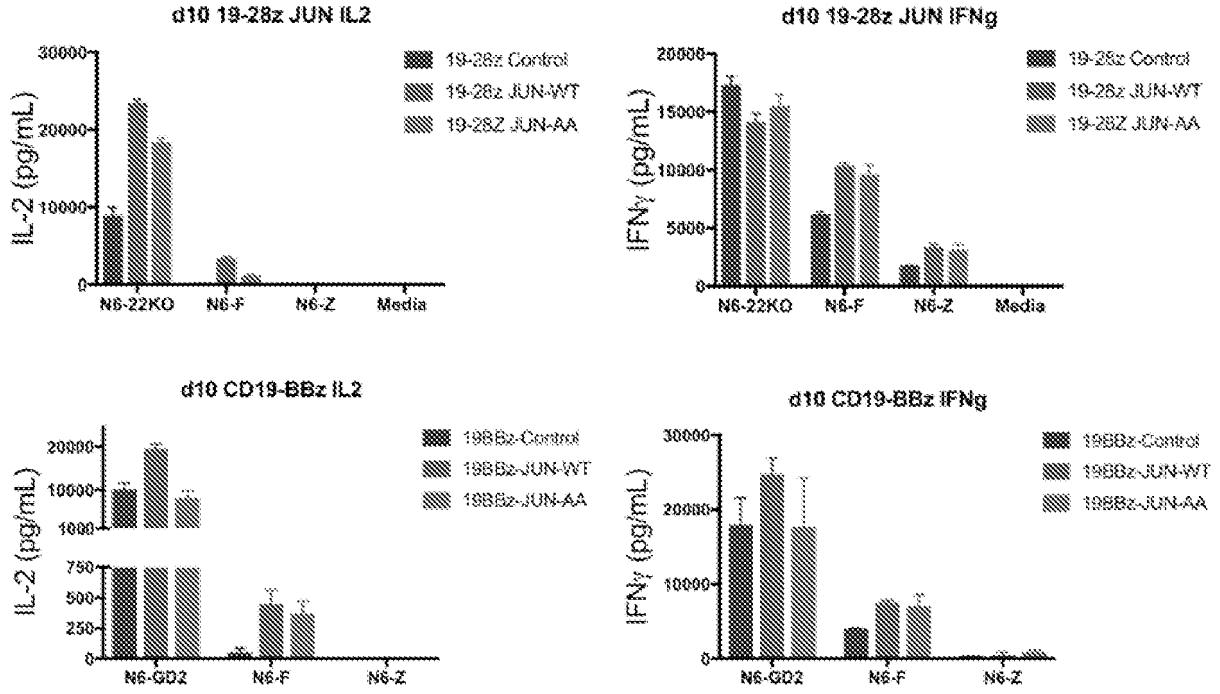


FIG. 29B

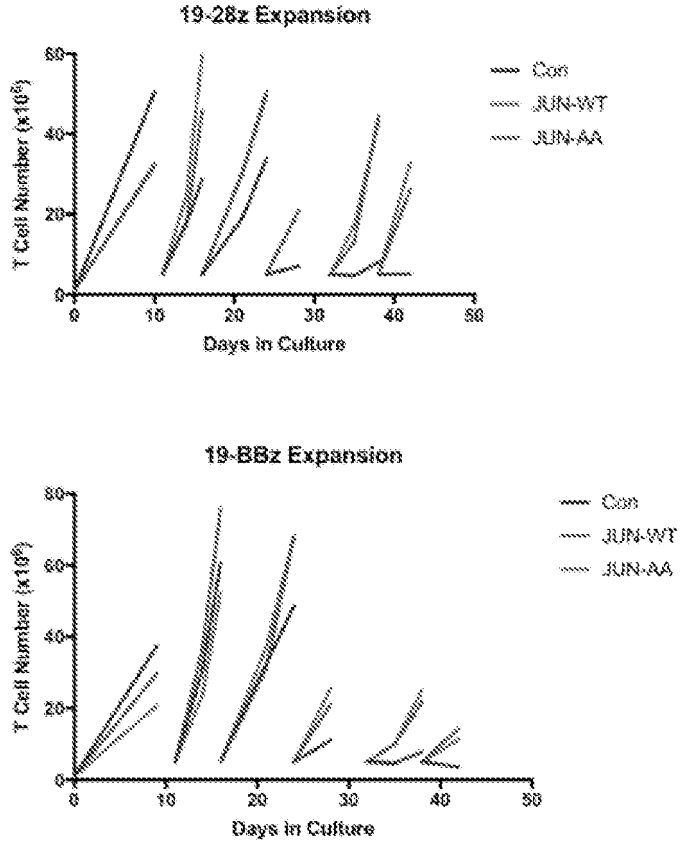


FIG. 29C

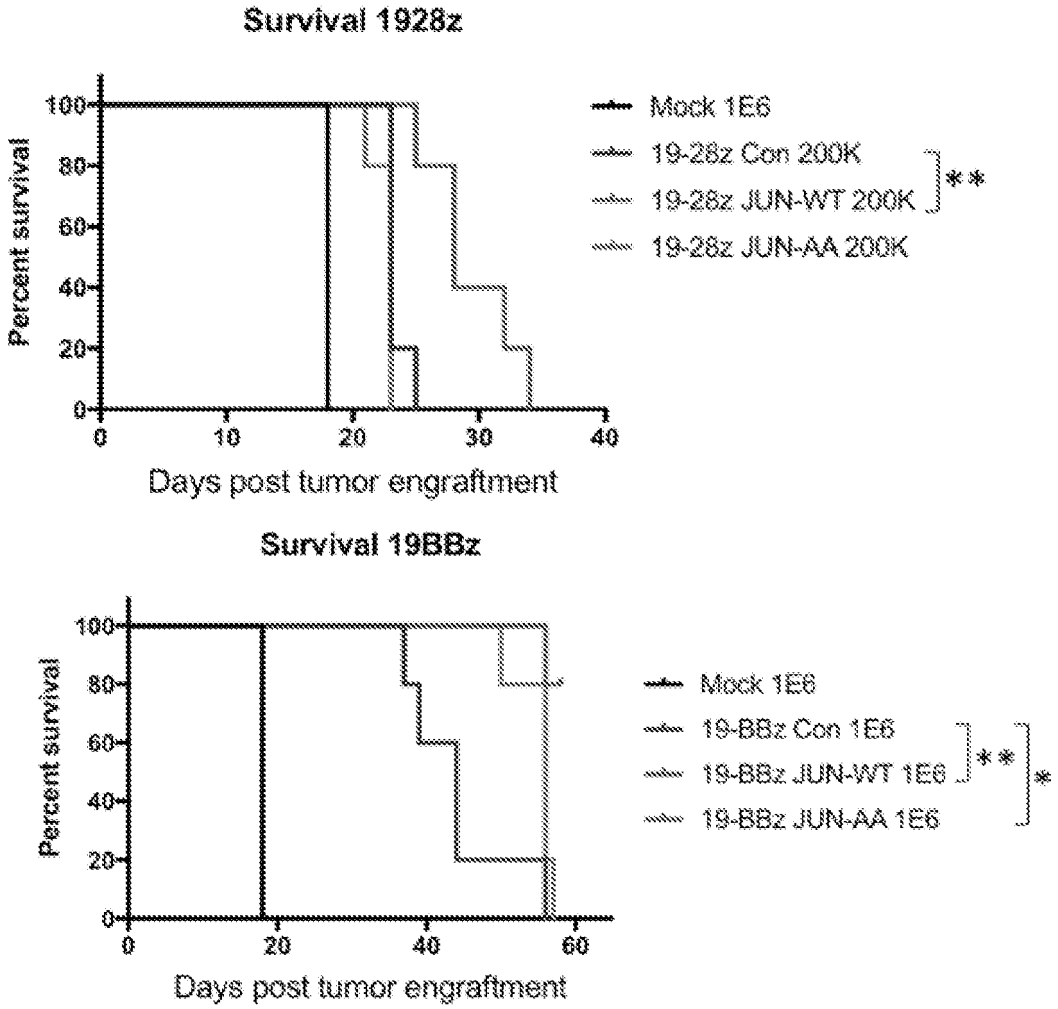


FIG. 30

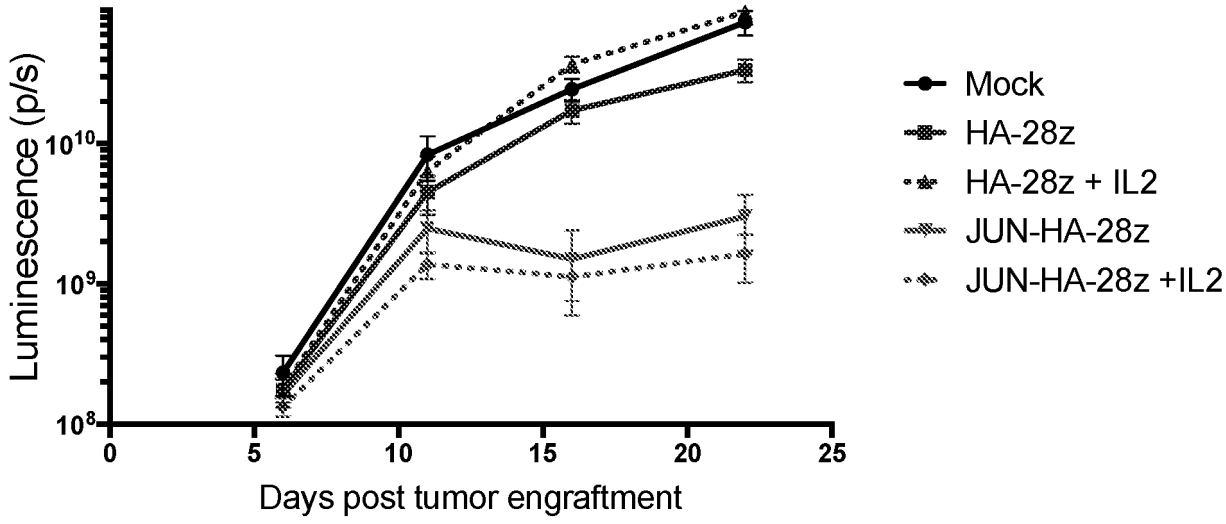


FIG. 31A

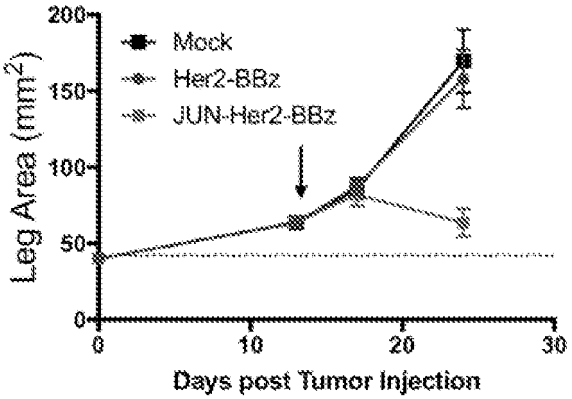


FIG. 31B

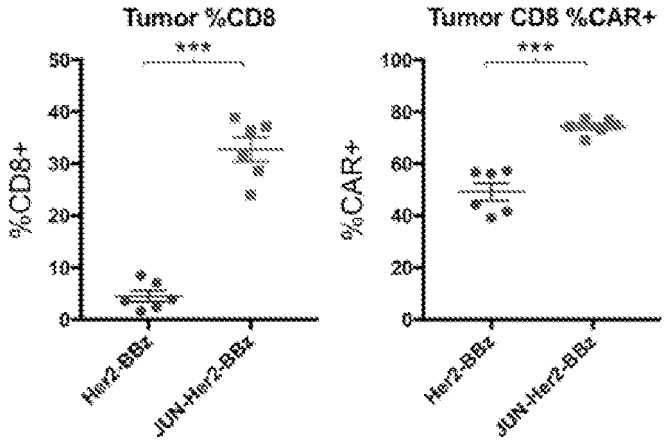


FIG. 31C

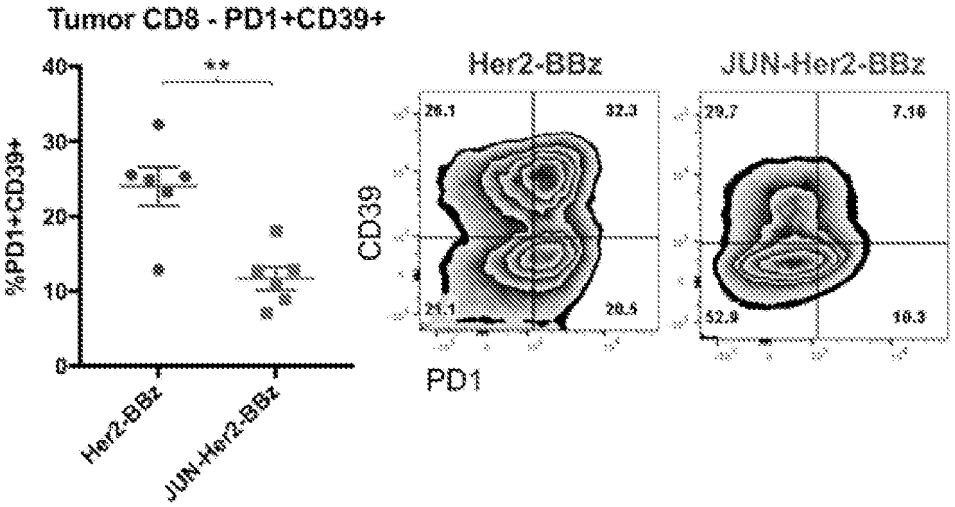


FIG. 31D

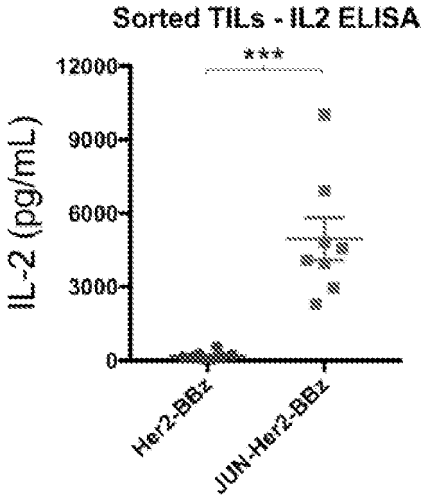


FIG. 31E

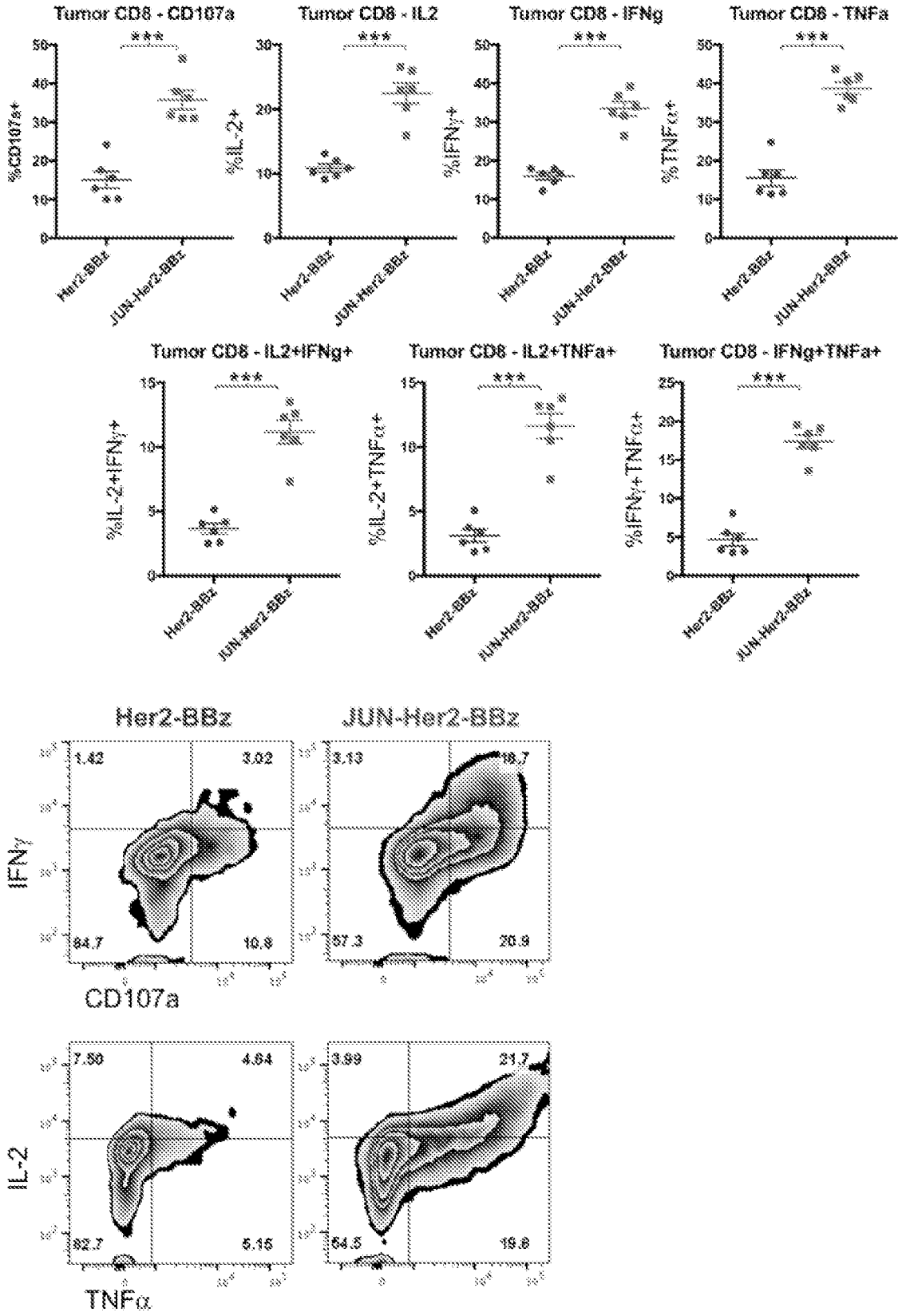
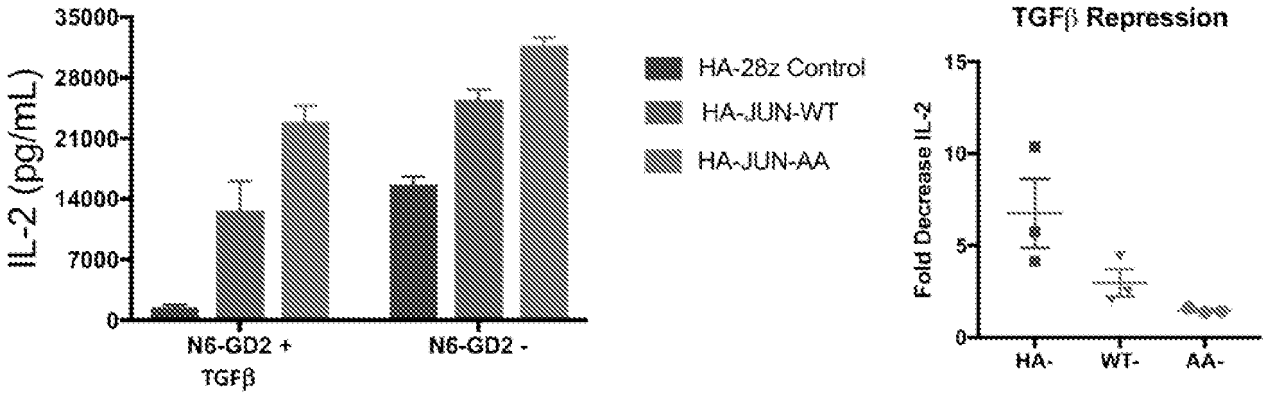

Tumor CD8 - CD107a
Tumor CD8 - IL2
Tumor CD8 - IFNg
Tumor CD8 - TNFa
%CD107a+
%IL-2+
%IFNγ+
%TNFα+
***
Her2-BBz
JUN-Her2-BBz
Tumor CD8 - IL2+IFNg+
Tumor CD8 - IL2+TNFa+
Tumor CD8 - IFNg+TNFa+
%IL-2+IFNγ+
%IL-2+TNFα+
%IFNγ+TNFα+
Her2-BBz
JUN-Her2-BBz
1.42
3.02
84.7
10.8
3.13
18.7
57.3
20.9
IFNγ
CD107a
7.50
4.64
82.7
5.15
3.99
21.7
64.5
19.8
IL-2
TNFα

FIG. 32


IL-2 (pg/mL)
35000
28000
21000
14000
7000
0
N6-GD2 + TGFβ
N6-GD2 -
HA-28z Control
HA-JUN-WT
HA-JUN-AA
TGFβ Repression
Fold Decrease IL-2
15
10
5
0
HA-
WT-
AA-

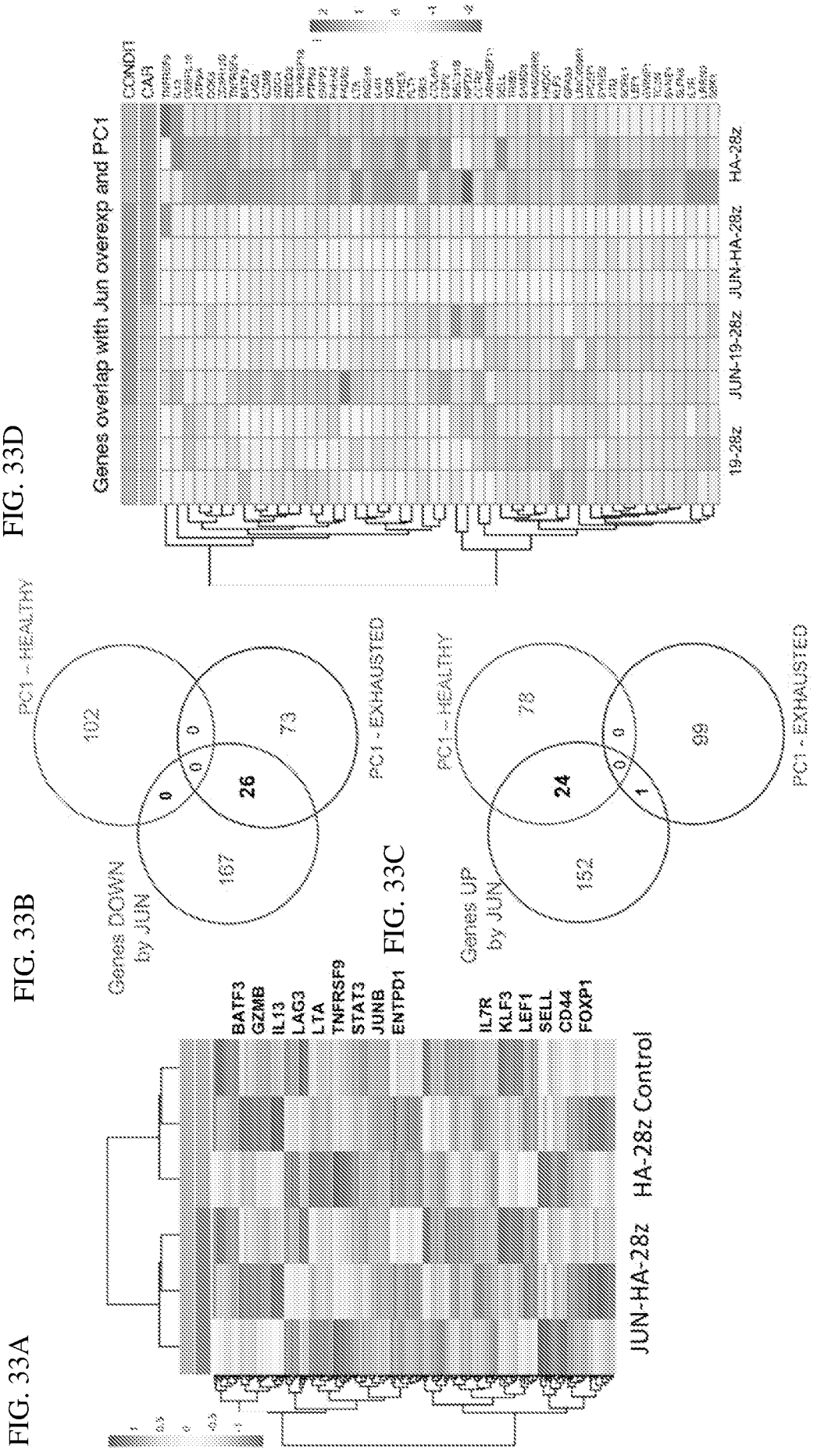


FIG. 33A

FIG. 33B

FIG. 33C

FIG. 33D